Volume LXXI Number 2

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

FEBRUARY 1921

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EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

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THE
BOTANICAL GAZETTE

FEBRUARY 1921

A CHEMICAL AND PHYSIOLOGICAL STUDY OF MOTTLING OF LEAVES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 277

F. M. SCHERTZ

(WITH SIX FIGURES)

Introduction

During the year 1917 SAMPSON (43) used *Coleus Blumei* (var. Golden Bedder) for studying the chemistry and physiology of leaf fall. He noticed that when the leaves were ready to drop they had completely or almost completely lost their chlorophyll, and that in general they were inclined to lose their chlorophyll or to mottle. It was thought that this accentuated material might be excellent for the study of the factors involved in mottling of leaves in general.

Loss of chlorophyll from plant organs is a very general phenomenon. It is thought of as an orderly and natural thing in autumnal coloration, but is considered a diseased condition when it occurs during the growing season. In the latter case it is spoken of as mottling or chlorosis. It is not at all improbable that a study which throws new light on mottling will also illuminate autumnal coloration. Indeed, this work indicates that the two have many things in common.

BORESCH (6) found that algae growing for some time on nutrient solutions gradually changed from a dark green to gold or red brown. If nitrate solution was added to these cultures, the algae regained

their normal green color. Extracts made from the algae showed that chlorophyll decreased while carotin increased in the algae as they browned. ARTARI (2) grew algae in the dark and found that with an organic source of nitrogen they remained green, but with potassium nitrate as the source of nitrogen the algae lost their green color. With potassium nitrate as the source of nitrogen the colorless algae would regain a normal green when placed in the light.

SWART (48), working on spring and autumn leaves, found that just as the leaves were ready to fall they usually became yellow, and this was accompanied by a decrease in protein, nitrogen, phosphorus, and potassium. WILLSTÄTTER (53) noticed that in autumn, as the leaves yellowed, their chlorophyll content became less.

BRIGGS, LYMAN, JENSEN, and MCLANE (7) have summarized the suggested causes of mottling in various plants. Excess of lime, magnesium, organic matter, or some essential element, deficiency in lime, iron, organic matter, or some essential element, low humus, high nitrogen, inorganic manures, frost, poor drainage, wind, sunlight, irregular supply of plant food and moisture, fungi or bacteria, nematodes, a filterable virus, and other causes are claimed by some to produce mottling. Chlorophyll may disappear owing to the absence of some essential constituent in the leaf, or to the presence of some deleterious substance. They thought that the soil was the cause of the mottling of citrus leaves and consequently analyzed it. It was found that mottling diminished as the humus ratio increased. Most of the trees which bore mottled leaves grew in soil which had a low nitrogen content. They believed that the mottling of the orange trees was definitely correlated with the low humus content of the soil, for mottling diminishes as the humus content increases. Alfalfa and bean straw were recommended for use in mulching the citrus trees.

JENSEN (25) analyzed green and mottled leaves, since it is known that organic matter attacks the soil minerals and sets free Ca, Fe, Mg, and PO_4, because these elements are closely connected with the formation of chlorophyll. It was found that badly mottled leaves from orange and lemon trees always contained a higher percentage of iron, calcium, magnesium, and phosphorus than the

healthy green leaves. Leaves in the medium stage of mottling sometimes contained more and sometimes less of these four elements. Midribs of the healthy leaves contained less of these elements than the mesophyll, while in badly mottled leaves the midribs contained more calcium and more phosphorus. The petioles contained less iron, calcium, and magnesium than either midrib or mesophyll in healthy and mottled leaves, while in badly mottled leaves the petioles contained more iron, calcium, and magnesium than either the midrib or mesophyll. Old leaves were found to contain more calcium and magnesium than new leaves. The yellow spots in the mottled leaves contained less calcium, magnesium, and phosphorus than the green parts of the same leaf. In working on the golden privet JENSEN found that the yellowed leaves contained more iron and 2.5 times as much phosphorus as the green ones. The increased amount of iron, calcium, magnesium, and phosphorus in the conducting tissues of the badly mottled leaves indicated that there was difficulty in the transfer of these materials to and fro.

MCBETH (37) found that plots receiving large applications of commercial fertilizers generally bore trees with badly mottled leaves, while trees receiving no nitrogen or barnyard manure generally showed little mottling. In other groves extreme mottling was frequently associated with a high nitrogen content. Moisture and nitrogen content of the mottled leaves were found to be higher than in the normal green leaves. His work seemed to indicate that too much nitrogen caused the mottling of citrus trees.

Investigation

The mottling of *Coleus* leaves occurs in a regular manner, proceeding from the edge inward and toward the base of the leaf. The edge usually yellows first, while only in rare cases do yellow spots develop in the central part before the edges become yellow. The basal portion seems to retain its green color longer than the tip, and the veins or the region near the veins are the last to lose their green color. The leaves on plants in good soil often die at the tip about the time of mottling, while the leaves on plants in poor soil rarely show this characteristic. Usually the leaves from plants

in good soil only partially yellow before they drop, while the leaves on plants in poor soil always completely yellow before dropping. This might suggest that mottling and dropping involve different factors.

MEYER (38) points out that leaves of *Tropaeolum* passed through the following stages: dark green 25 days, green 6 days, bright green 12 days, yellow green-yellow 3 days, and then bright yellow. The young leaves at the top of the stem were dark green, while those at the bottom were yellow or wilting. The yellowing he believed to be due to the aging of the leaves. The change from bright green to yellow green was very rapid, and took place in much the same manner as that described for *Coleus*.

MORPHOLOGICAL EXAMINATION

In comparing microscopically the green with the mottling leaf, several striking differences were observed. In the green leaf the chloroplasts were large and blue-green, and one to three or more starch grains were clearly visible in the chloroplasts. The guard cells seemed to retain their coloring matter longer than the adjoining cells. In the mottled leaf the chloroplasts were yellowish, fewer in number, much smaller, without a green tint, and without starch grains. The chloroplasts were clustered about the apparently normal nuclei or distributed throughout other parts of the cells. Also the general appearance of bacteria being active here was observed, and will be discussed later. The chloroplasts in the palisade cells of the normal green leaves were 2–5 μ in diameter, while those in the mottled leaves were 1 μ or less in diameter. The chloroplasts in the guard cells of the green leaves, as well as those in the mottled leaves, were about 1 μ in diameter. SWART observed that in the aging of leaves the chloroplasts broke down and the starch disappeared, but the nuclei and the plasma layers remained. He was not certain whether the chlorophyll escaped from the cell or not. The chloroplasts of the deep green leaves of *Tropaeolum majus*, as noted by MEYER, were larger than those of the pale green leaves. Since he made some very accurate determinations of the sizes of the chloroplasts and correlated the size with the color of the leaves, it will not be out of place here to quote him rather fully.

MEYER found that the protein of the palisade cells is located chiefly in the chloroplasts, which he looks upon as the birthplace of the proteins, hence one can see why the color of the leaves and the protein content are so intimately related. He observed also

TABLE I

COMPARISON OF CHLOROPLASTS OF PALISADE CELLS IN *Tropaeolum*

Color of leaves	Deep dark green	Deep green	Green	Bright green	Yellow
Relation of diameters....	126	100	86	72	52
Relation of volumes.....	200	100	64	38	14

that as the leaves yellowed there was little change in the size of the nucleus, the nucleolus, or in the protein content of the cytoplasm. He inferred that the formation of chlorophyll in the chloroplasts follows the development of protein in the leaves.

CULTURES

The plants were grown in the purest fine quartz sand, in new 4-inch flower pots. Experiments were conducted in which the sand was watered with nutrient solutions bearing all of the necessary elements for plant nutrition, or lacking either Ca, Mg, P, Fe, or N. Twenty-six plants were used in each of the six sets, making 156 in all. The set of plants receiving the complete nutrient was watered with Pfeffer's solution as given by DUGGAR (**14**). When iron, calcium, magnesium, phosphorus, or nitrogen was omitted from the complete solution, the salts suggested by DUGGAR were substituted. In the solution lacking iron, $Ca(NO_3)_2$, KNO_3, $MgSO_4$, KCl, and KH_2PO_4 were used; in that lacking magnesium, KNO_3, $Ca(NO_3)_2$, Na_2SO_4, $FeCl_3$, KH_2PO_4, and KCl; in that lacking phosphate, $Ca(NO_3)_2$, KNO_3, $MgSO_4$, KCl, and $FeCl_3$; in that lacking nitrate, $CaCl_2$, KCl, $MgSO_4$, KH_2PO_4, KCl, and $FeCl_3$; in that lacking calcium, $NaNO_3$, KNO_3, $MgSO_4$, KH_2PO_4, KCl, and $FeCl_3$. All of these were made up according to DUGGAR. Cuttings were made of the plants, which were then rooted in sand for two weeks. The freshly potted plants were watered first with nutrient solutions and then every morning with distilled water. About every week another application of the nutrients was made. The plants were

grown from May 1 to August 1. The effect of lack of nitrogen was evidenced in four or five days by noticeable yellowing.

Fig. 1 shows the condition of the various cultures on August 1. The plants with complete nutrient solution, and those without calcium, magnesium, or iron, grew about equally well. There was evidently enough of each of these elements already in the cuttings to care for considerable additional growth. All these plants had a good green color, indicating a plentiful chlorophyll supply. The effect of the absence of PO_4 or NO_3 was especially striking. A considerable nitrogen and phosphate supply evidently was necessary

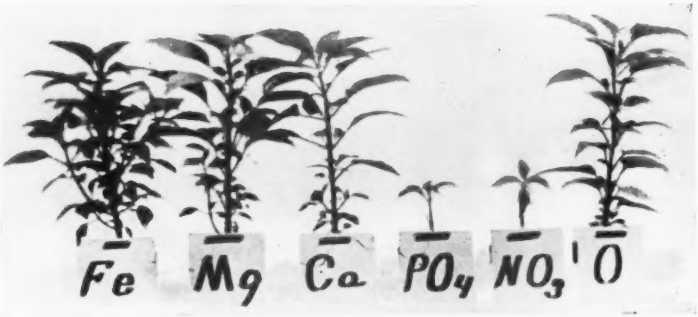

FIG. 1.—*Coleus* plants grown in various nutrient solutions: —Fe, iron lacking but all other essential elements present; —Mg, magnesium lacking, etc.; —O, all essential elements present; notice dwarfed condition of plants lacking phosphate or nitrate.

to cause any increment in the growth of the plants. The plants grown without PO_4 were very small, but the leaves were a deep green, even greener than any of the plants in the best soil. While addition of phosphorus was needed for any considerable growth of the plant, it was not needed for the maintenance of the chlorophyll. The deep green in the phosphorus-lacking plants probably was due to the high nitrate supply in proportion to the size of the plant, for here, as in all of this work, nitrate supply or its deficiency seemed to determine the development or the disappearance of chlorophyll. The plants grown in solutions lacking one of the elements Ca, Mg, or Fe, and those in complete nutrient solutions

all showed branches developing in axils of the leaves. The branching was especially prominent in the plants watered with nutrient solution lacking iron. No branches developed on the plants watered with nutrient solutions lacking PO_4 or NO_3.

On August 1 (3 months after planting) the number of pairs of leaves still attached was counted and compared with the number of pairs which had fallen. The plants were similar when the experiment was begun. Each group contained 26 plants and the average of these was taken.

Table II shows that when phosphate or nitrate was lacking a greater percentage of the leaves fell compared with any other element. This may partially be accounted for because plants lacking NO_3 or PO_4 had a smaller percentage of new leaves, and

TABLE II

EFFECT OF NUTRIENT ON LEAF FALL

LEAVES	ELEMENT LACKING					
	Fe	Mg	Ca	PO_4	NO_3	None
Average number of pairs dropped per plant.....	7	7	6	7	5	6
Average number of pairs still attached.....	9	9	9	3	4	9
Percentage dropped.....	45	45	40	70	56	40

consequently a smaller percentage of leaf fall. This did not account for the fact that when phosphate was lacking 14 per cent more of the leaves fell than when nitrate was lacking. The plants lacking Fe and those lacking Mg during the course of the experiment grew 16 pairs of leaves; those lacking Ca and those on complete nutrient solution each grew 15 pairs of leaves; those lacking PO_4 grew 10 pairs of leaves; and those lacking NO_3 grew only 9 pairs.

To 8 of the plants (2.5 months old) which were grown in sand cultures with NO_3 or PO_4 lacking, one watering was made with a solution which contained the lacking element. The effect is shown in fig. 2. Two weeks after the watering the height of the treated plants was about twice that of those which had no nitrate or no phosphate added, and the area of the new leaves put forth was from three to four times the area of the old leaves below them.

The plants to which nitrate was added showed noticeable greening in 4 or 5 days, and somewhat later became a normal green. Not only did the new leaves put forth become green, but even the light yellow leaves which were on at the time the nitrate was added became green.

Another set of experiments was carried out in which the elements Mg, N, P, Ca, and Fe were added to plants growing in pots in ordinary potting soil (figs. 3, 4). The solutions used were 2 per cent $FeCl_3$, 1 per cent $MgCl_2$, 6 per cent $CaCl_2$, 2 per cent KH_2PO_4, and 8 per cent $NaNO_3$. Eight plants were used for each treatment and 8 for controls, making 48 in all. In each case 0.25 cc. of the salt solution, diluted to 6.25 cc., was applied to the soil in the pots three times a week. In addition to this the plants were watered daily with tap water. The plants to which iron was added were given two or three drops of the iron solution each week. Some of the plants before being placed under treatment had already begun to mottle, but those to which nitrate was added rapidly regained their normal green. All of the plants to which nitrate was added retained their normal green and held their leaves better than the other cultures; also they branched and were sturdier than the rest. The plants shown in fig. 3 were grown 4 months in 2-inch pots, while those shown in fig. 4 were grown 4 months in 3-inch pots. Plants which were used as controls did not seem to do as well as the others, while the plants which received phosphate lost a large percentage of their leaves. The plants which received magnesium, calcium, or iron grew about equally well. The data given in table II show that lack of phosphate seemed to cause leaf fall, while here its addition caused the same effect. The lack of phosphates caused the leaf to fall, while the addition of phosphates alone to the soil, the nutrients not being present in a balanced ratio, produced the same effect.

DICKSON'S (12) work on oats is of interest in this connection. He found that oat seedlings grown in solutions deficient in phosphorus or nitrogen produced but one slender shoot. Plants grown in solutions deficient in Ca or Mg stooled heavily before those grown in complete nutrient solutions, and later the plants grown in solutions deficient in magnesium showed marked striping

FIG. 2.—Plants 3 months old: one labeled $-NO_3$ grown full time with NO_3 lacking in nutrient solution; one labeled $+NO_3$ grown with NO_3 lacking in nutrient solution for 2.5 months and then one dose of NO_3 added, after which plants grown for 2 weeks more; plants labeled $-PO_4$ and $+PO_4$ similarly treated; here PO_4 was substance lacking or added.

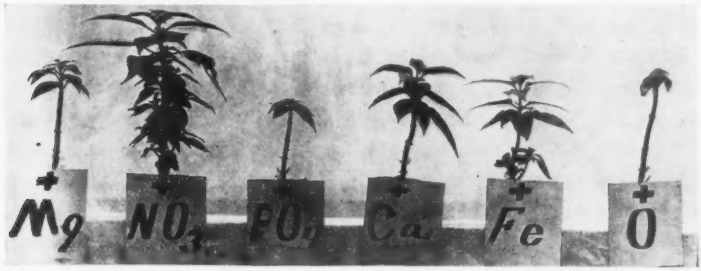

FIG. 3.—Plants 4 months old, during which time solutions containing Mg, N, P, Ca, Fe, or nothing were added to soil in respective pots; effect of addition of N shown by greater development of plant, as well as healthier color of leaves.

FIG. 4.—Plants similar to those in fig. 3, but grown in pots 1 inch larger

as the chlorophyll disappeared between the veins. Leaves and stems of plants grown in culture solutions deficient in phosphorus were purplish green. This color is apparently due to the presence of a purplish colloidal substance (perhaps a result of the decomposition of the chlorophyll) intermixed with the chlorophyll. Oat plants grown in solutions weak in nitrogen produced narrow purplish green leaves. Microscopical examination showed the chloroplasts more or less disorganized. A deficiency in phosphorus or in nitrogen produced a markedly unfavorable effect by causing a great decrease in the vegetative growth.

JOST (**27**) asserts that two *Helianthus* plants growing to maturity in three months used 1.4 gm. of KNO_3. These experiments with *Coleus* plants showed that one *Coleus* plant growing in soil in a 3-inch pot used the equivalent of 1 gm. of KNO_3, in addition to the nitrate which was in the soil, and then did not reach maturity. This shows that *Coleus*, being a plant much smaller than the sunflower, seems to use large quantities of nitrate. During the course of the experiment, if the nitrogen supply was discontinued at any time, the plants began to mottle at the margin of the leaves, but the leaves greened when the supply was again added. This shows that the plant was using the nitrate which was added to the soil in the pot. BURD (**9**) computed from the crop the amount of nitrate one barley plant used, and found that about 1.1 gm., calculated as potassium nitrate, was sufficient to bring the plant to maturity. PALLADIN (**40**) states as follows:

> Carbohydrates are essential to the formation of chlorophyll. Plants fall into two groups according to the carbohydrate content of their etiolated leaves. In one group (for example, barley) the etiolated leaves contain much soluble carbohydrate material, while in the other group (as beans and lupines) the etiolated leaves contain very little carbohydrate. If etiolated leaves of these plants are removed and floated upon water in the light, those of barley become green, while almost all the bean leaves and all those of lupine remain yellow. If the latter are floated, not upon water but upon a saccharose or glucose solution, then they also become green.

The writer conducted experiments with *Coleus* leaves similar to these of PALLADIN. Young *Coleus* plants were kept in darkness until the leaves became etiolated. These etiolated leaves of *Coleus* when floated on distilled water in the light remained yellow,

those on KNO_3 solution died, while those on a 5 per cent sugar solution greened slightly. On the other hand, *Coleus* leaves which had mottled in the light did not green again when floated on any of these solutions. In the case of etiolated *Coleus* leaves it apparently is true that they do not form chlorophyll because of the lack of carbohydrates. The case of the mottled leaves cannot be explained on this basis. The catalase experiments (see later) show that the vigor of the mottled leaves is greatly reduced, and this would account for the inability of the mottled leaves to green again. In the case of mottled leaves carbohydrates are evidently not the limiting factor, for tests for carbohydrates showed that plenty of starch was present in the leaves.

Microchemical analysis

The differences between the normal green and the mottled leaf were determined by microchemical methods. The tests used were those given by MOLISCH and TUNMANN in their texts on microchemistry, and generally several tests were applied in order to determine the presence or absence of a substance.

Tests for starch were made upon the two types of leaves. Green and mottled leaves gathered before 8 o'clock (sun time) showed a wide variation. The guard cells of the mottled leaves were especially full of starch, while some starch was found in the other parts of the leaves; the whole leaf had a bright blue color after testing with iodine. Much starch was found in all parts of the green leaf, and it was colored deep violet to black by the iodine used. In the evening, after a bright sunny day, leaves were collected and the amount of starch again determined. The guard cells of the mottled leaves were well filled with starch, and the other cells had much more starch than they had in the morning. The chloroplasts which were present appeared to be active in forming starch, even though no chlorophyll seemed to be present. Since this investigation the writer has had occasion to make some very accurate tests for small amounts of chlorophyll, and it is doubtless true that if solutions of the pigments of mottled leaves of *Coleus* had been subjected to spectrophotometric tests, chlorophyll would have been discovered, at least in small amounts. All cells

of the green leaf were completely filled with starch, which was present in larger grains than it was in the mottled leaves. The masses of starch here appeared to be about five times the diameter of the masses in the mottled leaves. At noon, a healthy plant which had the lower leaves mottled was placed in the dark. Immediately one-third of the tip end of one of the green leaves was cut off, then in 6 hours another one-third was cut off, and at the end of 18 hours the remainder of the leaf was removed. The same was done with the mottled leaf.

Table III shows that the translocation power of the leaf was still active, and proves that diastase was not inhibited by oxidizing enzymes, as was believed by WOODS (18) in the case of mosaic leaf of tobacco.

TABLE III

TRANSLOCATION OF STARCH

Leaf	Placed in darkness	After 6 hours	After 18 hours
Green	Much	Medium	Little
Mottled	Fair amount	Less starch	Minute traces

Tests for iron in the chloroplasts were made with potassium ferrocyanide. Both the green and the mottled leaves had iron in their chloroplasts. The chloroplasts which were present in the mottled leaf were colored about as deeply as those in the green leaf. From the blue tint which was produced in the leaves, the green ones appeared to have more iron than the mottled ones. From the macrochemical results which follow, it is evident that some of the iron in the leaves is "masked."

In comparing the amount of ammonium magnesium phosphate crystals which were formed in the two leaves on the addition of sodium ammonium phosphate, there appeared to be slightly more crystals formed in the cells of the mottled leaf blade. In both green and mottled leaves less magnesium was found in the upper part of the petiole than in the part of the petiole nearest the stem. The petioles of young green leaves had about the same amount of magnesium as the petioles of strongly mottled leaves. In the petioles about one crystal of ammonium magnesium phosphate

per cell was formed in the cortex and in the pith. In the plant with mottled leaves 2 or 3 crystals were formed in each pith cell of the stem. Magnesium was also present in the xylem, phloem, cortex, and epidermal cells.

In making comparative tests of the leaves for calcium, apparently a few more calcium sulphate crystals were formed in the cells of the mottled than the green leaves. In the green leaves much calcium was found in the epidermis of the petioles, some in the xylem and phloem regions, and little in the parenchyma. The same was true of the mottled leaves. In the stems many calcium sulphate crystals were formed in the pith cells on the addition of H_2SO_4. If there was any difference, more crystals were formed in the stems of the mottled plants. Calcium sulphate crystals were also formed in the xylem, phloem, cambium, and cortical regions. The fact that the mottled leaves and stems were always older than the green leaves and stems of the same plant would account for more crystals being formed in their cells.

In testing for phosphates by the addition of ammonium and magnesium chloride, only a very few crystals of ammonium magnesium phosphate were formed in the green leaves or in the mottled ones. Evidently the phosphorus must have been in some organic form in which it is not readily reactive with the reagents used, hence no conclusion can be drawn from this test regarding the metabolic disturbances which may be produced by it.

The test for nitrates gave the most interesting result. All of the green leaves gave tests which showed that an abundance of nitrate was present, while no positive results were obtained from the completely mottled leaves. It is of value to compare the progress of the mottling of the leaf with the absence of nitrates. The first signs of mottling usually appeared at or near the lobes of the leaf, and it was here that the test for nitrates was first negative. At this stage the greatest amount of nitrates was found in the conducting tissues of the leaf. Also in the deepest green leaves the conducting tissues contained the most nitrate. As the green disappeared from the tip of the leaf, more and more nitrates were found only in the veins close to the base. At this stage only a little greenish tint remained in the leaves. Usually as long as the

veins still showed a greenish tint some nitrates were found to be present. The last traces of nitrate in the leaf were found only in the petioles. In a moderately yellowed plant whose leaves were very slightly green, nitrates were found only in the pith region at the base of the stem; hence nitrates began to disappear at the very tip of the leaves and were last found only at the base of the stem. The nitrates disappeared last in the storage regions.

Among other workers in microchemistry, SWART (**48**) found that in yellow leaves in autumn the amount of phosphorus, nitrogen, and potassium decreased shortly before the leaves fell. Comparing this with the mosaic disease of tobacco, FREIBERG (**18**) reports that more proteins were present in the lighter areas of the leaves than in the darker. Nitrates were present in about the same quantities in healthy and diseased areas. Ammonium salts, iron, calcium, magnesium, potassium, phosphorus, and sulphur were also present in the same quantities in the chlorotic and in the dark green areas. By employing Folin's micro-Kjeldahl method less nitrogen was found in the dark areas than in the lighter diseased areas. Diseased areas of the tobacco leaf gave a more pronounced reaction with Millon's reagent, the xanthoproteic reaction, and the biuret test than did the healthy areas. More carbohydrates were always present in the dark green or healthy areas.

Macrochemical analysis

Since the whole leaf of the plant mottled completely, it was easy to compare the green with the mottled leaves by an analysis of the leaves, including the petioles, for the presence or absence of the substances which were suspected of causing the disturbance. In making the following analyses, controls were always run on a known sample, and in many cases several methods of analysis were tried and the one which gave the best theoretical results was used.

In estimating the amount of iron present in the leaves, the method described by MARRIOTT and WOLF (**34**) was used. The blade, petioles, and region of the abscission layer were analyzed separately. A piece 3–4 mm. long was used for the analysis of iron in the abscission layer, and the petiole was cut off at the base

of the leaf. The leaves were taken from the same plants, which were grown in ordinary potting soil, dried to constant weight at 100° C., and then the dry material ashed at a low red heat. The iron was calculated as free iron.

The analysis for iron showed that the amount of iron increased in the abscission layer, in the petioles, and in the leaf blades in

TABLE IV

COMPARISON OF AMOUNTS OF IRON IN GREEN AND MOTTLED LEAVES

Material	Wet weight	Dry weight	Percentage dry weight	Grams of iron	Grams of iron per gram of dry weight	Grams of iron per 100 grams of dry weight (average)
	Mottled leaves					
Abscission layer....	0.3450	0.0200	5.79	0.000,016	0.000,80	0.108
	0.2585	0.0146	5.65	0.000,014	0.000,98	
	0.2660	0.0166	6.24	0.000,024	0.001,47	
Petioles.....	2.0320	0.0865	4.25	0.000,042	0.000,49	0.042
	1.5550	0.0582	3.74	0.000,036	0.000,62	
	1.4080	0.0588	4.17	0.000,009	0.000,15	
Leaf blade.....	8.5660	0.4930	5.75	0.000,81	0.001,64	0.130
	5.4694	0.3008	5.50	0.000,37	0.001,21	
	6.4706	0.4520	6.98	0.000,48	0.001,06	
	Green leaves					
Abscission layer....	0.6450	0.0300	4.65	0.000,02	0.000,666	0.079
	0.4224	0.0206	4.87	0.000,02	0.000,970	
	0.3980	0.0192	4.82	0.000,02	0.000,729	
Petioles.....	4.7160	0.1690	3.58	0.000,045	0.000,266	0.030
	2.4650	0.0992	4.02	0.000,038	0.000,383	
	1.5780	0.0988	6.26	0.000,024	0.000,230	
Leaf blade.....	17.4220	1.2100	6.94	0.000,47	0.000,388	0.073
	8.1016	0.5575	6.88	0.000,39	0.000,699	
	10.3500	0.8080	7.80	0.000,90	0.001,110	

the mottled leaves. This result disagreed with the microchemical report, perhaps owing to the fact that the iron was bound in some way and was released only by ashing the leaf. It showed that about 1.55 per cent of the ash of the green leaves was Fe_2O_3. According to PALLADIN (**40**), beech leaves have 2.30 per cent of Fe_2O_3 in the ash, while JOST (**27**) states that tobacco leaves have 1.95 per cent. According to PALLADIN 0.09 per cent of the dry weight of pea

leaves and 0.11 per cent of the dry weight of bean leaves was Fe_2O_3, while *Coleus* leaves had 0.23 per cent as Fe_2O_3. The Fe_2O_3 in beech leaves increased from 0.8 per cent of the ash in May to 1.3 per cent of the ash in October. One concludes that the mottling of the *Coleus* leaves was not due to a deficiency in iron, for at all times the leaves had enough iron, when compared with

FIGS. 5, 6.—Fig. 5, plants from which leaves were taken for analysis: leaves missing on upper part of plant at left taken for analysis; lower leaves mottled and fallen off; lower pair of leaves still on each plant partially mottled; plant at left type *A*, plant at right type *B;* fig. 6, another group of plants from which leaves were taken for analysis: plant at right type *A*, plant at left type *B;* lower pair of leaves still on each plant partially mottled; plants grown close together to produce larger leaves, accounting for leaflessness of stems.

other plants, to carry on their metabolic activities in a normal manner. This fully agrees with the conclusions drawn from the cultures.

Two types of plants were used for the analyses which follow. Plants of type *A* were grown in 3- and 4-inch pots, until ready for repotting, and then put into 6- and 8-inch pots, using ordinary

potting soil for repotting. Plants of type *B* were taken from the same group as *A*, and were repotted in pots of the same size as *A*, but sand was used instead of soil. In this way the plants were grown under exactly the same conditions of light and moisture as were the plants of type *A*, but the amount of soil nutrient was considerably reduced. The larger plant in each figure (figs. 5, 6) is of type *A*. The two plants shown in fig. 6 were grown in 6-inch pots, while those in fig. 5 were grown in 8-inch pots. All the leaves designated "green" were picked from plants of type *A*, and the "mottled" leaves were picked from both sets of plants and were analyzed separately. The mottled leaves from plants of type *A* as a rule were only partially mottled, while those from type *B* were always completely mottled. The green leaves were picked from the plant 3 or 4 nodes above the yellowing leaves,

TABLE V

MAGNESIUM

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Per 100 gm. dry weight
Green	0.0922	0.0980	0.0952	0.0951	1.50
Mottled *A*	0.0883	0.0892	0.0935	0.0903	1.66
Mottled *B*	0.0938	0.0740	0.0840	0.0839	1.35

and the mottled leaves from types *A* and *B* were picked just about the time they were ready to fall. The leaves were always picked before 8:00 A.M. in order that the results might better be compared.

The amounts of magnesium and calcium were determined according to the methods given in Bulletin 107 of the Bureau of Chemistry (**8**). In each case 40 gm. of green material was used for the determinations, and the results are given in grams of the free element per 100 gm. of wet material. The leaves were dried at 100° C. before being analyzed, and the dry weights found were used in calculating the grams of calcium and magnesium per 100 gm. of dry weight.

The magnesium content of mottled *Coleus* leaves differed little from that of green ones, and the magnesium content of both was somewhat higher than that reported for other leaves. As calculated from table V, the ash of green *Coleus* leaves was 17.5

per cent MgO, while according to PALLADIN (40) that of beech leaves was 7.20 per cent MgO, and according to JOST that of tobacco leaves was 7.36 per cent MgO. PALLADIN states that 1.02 per cent of the dry weight of pea leaves was MgO, while 0.66 per cent of bean leaves was MgO. The writer found that 2.48 per cent of the dry weight of green leaves was MgO. In agreement with the results from cultures, the analytical data also indicated that the magnesium was in excess of the needs of the plant, and that decomposition of chlorophyll in these leaves was not due to a shortage in magnesium.

The amount of calcium present in mottled leaves of *Coleus* was slightly greater than that in green, and the calcium content of both was less than that found in tobacco and beech leaves, while it was more than that found in pea and bean leaves. As

TABLE VI

CALCIUM

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Per 100 gm. dry weight
Green	0.206	0.222	0.284	0.237	3.31
Mottled *A*	0.215	0.255	0.283	0.251	3.93
Mottled *B*	0.169	0.225	0.240	0.211	3.03

calculated from table VI, the ash of green *Coleus* leaves was 33.2 per cent CaO, while according to JOST that of tobacco leaves was 36 per cent CaO, and according to PALLADIN (40) that of beech leaves was 44.3 per cent CaO. PALLADIN found that 3.21 per cent of the dry weight of pea leaves was CaO, and of bean leaves 1.33 per cent was CaO. The increase of calcium (based on dry weight) from 3.31 per cent in the green *Coleus* leaves to 3.93 per cent in the mottled is easily accounted for by the fact that the older leaves have different ash constituents (44) from the young leaves. In beech leaves (40) the MgO content (based on dry weight) increased from 4.3 per cent in May to 5.6 per cent in July, and then decreased to 4.1 per cent in October. These data and the culture experiments showed that the amount of calcium present at all times was sufficient to care for the physiological needs of the plant.

Phosphates were determined by the NEUMANN-PEMBERTON (35) method and the result is given as free phosphorus. In each analysis 20 gm. of fresh leaves was used, and before being analyzed was dried at 100° C. in order to determine the dry weight.

The amount of phosphorus present in mottled *Coleus* leaves was considerably less than in green ones, and the phosphorus content of the green leaves was about the same as that of other leaves (tobacco and beech), but the phosphorus content of pea and bean leaves was higher than that of green *Coleus* leaves. As calculated from table VII, the ash of green *Coleus* leaves was 6.41 per cent P_2O_5, while according to PALLADIN (40) the ash of beech leaves was 7.80 per cent P_2O_5, and according to JOST the ash of tobacco leaves was 4.66 per cent P_2O_5. Of the dry weight of

TABLE VII

PHOSPHORUS

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of phosphorus per 100 gm. dry weight
Green	0.031	0.032	0.019	0.027	0.398
Mottled *A*	0.011	0.011	0.010	0.011	0.181
Mottled *B*	0.010	0.010	0.011	0.011	0.176

the green *Coleus* leaves, 0.91 per cent was P_2O_5, while according to PALLADIN pea leaves had 1.67 and bean leaves 2.19 per cent P_2O_5. With mottling the total percentage of phosphorus in the leaf of *Coleus* calculated as P_2O_5 (on ash basis) had decreased from 6.41 to 2.88 per cent, or somewhat over 50 per cent. In the case of beech leaves, PALLADIN found that in May the P_2O_5 content was 32.4 per cent of the ash, while in October it was only 5.1 per cent. The percentage decrease of P_2O_5 in *Coleus* leaves was not due to an accumulation of ash, but apparently to the decrease in the P_2O_5 before the leaf fell, for when one calculated the amount of P_2O_5 present in the green and in the mottled leaves, there was always a reduction of 50 per cent or more, whether the calculation was based on dry weight, wet weight, or ash. This reduction in the phosphate content of *Coleus* leaves was similar to that found by SWART (48) for other leaves, and in all probability the changes

which took place in *Coleus* are of a similar nature to those accompanying the yellowing of leaves in the autumn. Since the green and mottled leaves of *Coleus* were about the same size, and since there was only two or three weeks' difference at the most in the ages of the leaves of *Coleus*, it is hardly possible that the differences could be accounted for in any way except that the P_2O_5 content actually decreased, and this would mean a transfer of materials from the leaf to the stem. If the amount of phosphorus were figured per leaf, then, since the leaves were about the same size, there would be nearly a 50 per cent reduction in the amount of phosphorus. Such a conclusion is in harmony with results from the cultures, for they showed that phosphate was necessary for growth of the plant, and when phosphates were deficient a larger percentage of the leaves fell. This conclusion is not out of

TABLE VIII

PHOSPHORIC ACID CONTENT OF LEAVES OF *Acer Negundo*

Phosphoric acid	May 7	June 6	July 5	August 2	September 3	September 25
Percentage dry weight..	1.500	0.801	0.705	0.580	0.586	0.333
Grams per 200 leaves...	0.256	0.200	0.210	0.134	0.147	0.099
P_2O_5 as percentage of ash	20.8	10.7	8.8	6.3	6.2	2.9

harmony with the work of SCHULZE and SCHÜTZ (44). In working on *Acer Negundo* these investigators showed that the phosphorus content decreased gradually and quite definitely from May to September, whether the phosphorus content was calculated as percentage of dry weight, grams per 200 leaves, or calculated from the ash. Only the results of their work on leaves collected in the morning will be given here, as they are most directly comparable with those of the writer, yet it is worthy of note that nearly always the phosphorus content of the leaves in the evening was greater than in the morning. This seems to indicate storage and synthesis during the day and a loss of phosphorus compounds during the night. The phosphoric acid content of the leaves is summarized in table VIII.

This work shows that the amount of phosphoric acid in the leaves of *Acer Negundo* decreases as the season advances. From these data one cannot agree with WEHMER or RIESMÜLLER (as

cited by PALLADIN 41), who believe that the absolute amounts of phosphoric acid do not diminish as the season progresses. The writer must agree with SCHULZE and SCHÜTZ, who find that the phosphoric acid content decreases as the leaves age or as autumn approaches.

Further evidence for the correctness of these views is presented by TUCKER and TOLLENS (49), who have shown that phosphorus decreased markedly in the leaves of the plane tree on or about October 8. The amount of phosphorus in the leaves at the end of the growing season was less than 50 per cent of that found in the leaves during the earlier part of the year. They believe that the three plant nutrients (nitrogen, phosphorus, and potash)

TABLE IX

PROTEIN NITROGEN

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green	0.189	0.243	0.207	0.213	3.35
Mottled *A*	0.076	0.080	0.087	0.081	1.39
Mottled *B*	0.073	0.064	0.079	0.072	1.14

passed from the leaves into the stems as the leaves aged, and were not washed out by rains.

Protein nitrogen was determined by the Gunning-Arnold modification of the Kjeldahl process. In each case 10 gm. samples of the fresh leaves were used, and the results as given in table IX are grams of nitrogen per 100 gm. of fresh leaves.

The green leaves contained 1.33 per cent protein (N×6.25), while the mottled leaves contained 0.51 per cent, and those on poor soil *B* 0.45 per cent, when calculated on the wet weight of the leaves. Based on the weight of the dry leaves, the green ones contained 20.9 per cent, the mottled *A* contained 8.7, and *B* 7.13 per cent protein. PALLADIN found considerable variation in the percentage of total nitrogen and protein nitrogen between etiolated and green leaves of various plants, but the difference was not always in the same direction. OTTO and KOOPER, and LECLERC DU SABLON (19) found that leaves decrease in their protein content

from spring to autumn. It is evident that the protein content of green and mottled *Coleus* leaves does not parallel that of the green and etiolated leaves of PALLADIN. If it resembles the protein change with progress of the growing season, it is at least much more rapid. It is probably quite similar to the rapid changes just preceding leaf fall in autumn.

The work of SCHULZE and SCHÜTZ (**44**) may again be relied upon to show the normal changes which take place in *Acer Negundo*.

TABLE X

NITROGEN PRESENT IN *Acer Negundo* LEAVES AT VARIOUS TIMES OF YEAR

Protein N	May 7	June 6	July 5	August 2	September 3	September 25
In 200 leaves	0.734	0.973	1.211	0.864	0.791	0.628
Percentage in dry material	4.304	3.906	4.068	3.745	3.163	2.110

The magnitude of the changes in the protein content of *Acer Negundo* is in harmony with that of *Coleus*, except that the changes in the latter are much more precipitous.

MEYER (**38**) made macroscopic tests for proteins in the leaves of *Tropaeolum* by means of the xanthoproteic reaction. The natural color of the leaves was noted and compared with the depth of color which was produced by the xanthoproteic test. He found that as the green color of the leaf disappeared, the xanthoproteic reaction became less and less, or, in other words, as the protein of the chloroplast decreased, the chlorophyll in the chloroplast decreased also.

TABLE XI

XANTHOPROTEIC REACTION OF NORMAL ILLUMINATED GROWING LEAVES OF *Trapaeolum*

Dark green leaves	5 to 4*
Green	3 to 4
Bright green	3
Yellow green	2 to 3
Yellow	2
Bright yellow	1 to 2

*The larger the number the greater the amount of protein present.

In the leaves of the plane tree (*Platanus occidentalis*) TUCKER and TOLLENS found that the protein nitrogen decreased gradually

per unit area from July 15 until November 5. Over three-fourths of the nitrogen disappeared in this time.

In analyzing the leaves for total nitrogen when nitrates are present the method as described in Bulletin 107 (8) was used, with zinc as the reducing agent. Samples (10 gm.) of fresh leaves were used, and the results were calculated to grams of nitrogen per 100 gm. of fresh leaves. This analysis gives the total of the three forms (31) of nitrogen which may be found in organic matter (such as leaves), namely, nitrogen in a state of organic combination, nitrogen in ammonia or its combinations, and nitrogen in a more highly oxidized state as salts of nitrous or nitric acid. Table XII shows that there is a marked reduction in the total nitrogen content of the leaf with mottling. BONCQUET (5) found that the total

TABLE XII

PROTEIN NITROGEN+NITRATE NITROGEN+AMMONIUM SALTS

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green	0.283	0.267	0.249	0.266	4.19
Mottled *A*	0.117	0.107	0.103	0.109	1.87
Mottled *B*	0.074	0.071	0.060	0.068	1.09

nitrogen calculated on the basis of ash was always less in the diseased leaves than in the healthy ones.

The amount of nitrogen present in the leaves as NO_3 was determined by the Schlösing-Wagner method as given in Bulletin 107. For each determination 25 gm. of leaves was used. The leaves were finely ground in a mortar with quartz sand, boiled for 2 hours, made up to a definite volume, filtered through cheesecloth, and the amount of nitrate estimated in an aliquot part. The amount of gas as nitric oxide set free was then measured in the burette of a Van Slyke apparatus (35), and the nitric oxide was absorbed in a Hempel pipette containing NaOH and $KMnO_4$. The residual gas was then measured in the burette. The difference gave the amount of nitric oxide, which was reduced to standard temperature and pressure, and was calculated to grams of nitrogen per 100 gm. of wet weight, as given in table XIII, which shows an

enormous reduction in the percentage of nitrate nitrogen with mottling.

TABLE XIII

NITRATE NITROGEN

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green	0.004,28	0.002,78	0.002,68	0.003,25	0.0485
Mottled *A*	0.001,90	0.000,80	0.000,72	0.001,14	0.0106
Mottled *B*	0.000,05	0.000,10	0.000,05	0.000,07	0.0011

Table XIV summarizes the results given in tables IX, XII, and XIII, and shows the following changes in the nitrogen compounds of the leaf with mottling. When the green leaves of *Coleus* were

TABLE XIV

COMPARISON OF NITROGEN FOUND

Form of nitrogen	Percentage wet weight	Percentage distribution of the three forms of N	Percentage dry weight
	Green		
Protein nitrogen	0.213	80	3.35
N as ammonium salts	0.050	18.8	0.79
N as nitrate	0.003	1.2	0.05
Total	0.266	100.01	4.19
	Mottled *A*		
Protein nitrogen	0.081	74.3	1.39
N as ammonium salts	0.027	24.8	0.46
N as nitrate	0.001	0.9	0.02
Total	0.109	100.01	1.87
	Mottled *B*		
Protein nitrogen	0.072	.....	1.14
N as ammonium salts	−0.004	.....	−0.05
N as nitrate	0.0001	.....	0.0011
Total	0.068	.....	1.09

compared with the mottled, it was found that the amount of protein nitrogen, nitrogen as ammonium salts, and nitrogen as nitrates disappeared as the leaves mottled. The greatest decrease was found in the protein nitrogen, which showed that the protein

compounds were rapidly being broken down. In this connection SAMPSON (43) showed that the amino acid nitrogen of *Coleus* (based on dry weight) increased from 0.056 to 0.072 per cent as the leaves mottled. Table XIV shows further that when the leaves were completely mottled the nitrate nitrogen almost disappeared, which is in complete accord with the microchemical determinations. The table shows also that inorganic forms of nitrates were used up before the protein nitrogen was exhausted. PALLADIN shows that such was the case in starving plants. BONCQUET (4) believed that the plants he worked on were starving, due to lack of nitrogen.

Free ammonia was determined by a modification of the FOLIN method (35). Twenty-five gm. of the fresh leaves was finely ground with quartz sand, placed in an aeration tube, and ammonia-

TABLE XV

FREE AMMONIA

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Nitrogen per 100 gm. dry weight
Green	None	None	None	None	None
Mottled *A*	None	None	None	None	None
Mottled *B*	0.000,18	0.000,14	0.000,21	0.000,18	0.003,25

free air drawn through the tube for 2 hours. The ammonia which came from the leaves was absorbed in an aeration tube which contained 0.1N H_2SO_4. The ammonia set free was distilled off, after adding NaOH, and Nesslerized (33). Especial care was taken to use materials absolutely free from ammonia. The ammonia is calculated as grams of nitrogen per 100 gm. of wet weight. For the analysis leaves were selected which were free from mechanical injuries or drying at the tips. The mottled leaves *A* still had some green in them, while those of *B* were wholly yellow.

Other forms of ammonia were determined, such as albuminoid ammonia. The method as outlined by MASON (33) was followed, in which 10 gm. of the fresh leaves was distilled in the presence of NaOH and $KMnO_4$, 600 cc. of distillate distilled off and then a portion of it Nesslerized. The results calculated as grams of

nitrogen per 100 gm. of wet weight show that the mottled leaves had much less of the albuminoid ammonia.

Distillation of the leaves with Na_2CO_3 was also undertaken. Twenty-five gm. of the leaves was distilled in a flask with 2 gm.

TABLE XVI

ALBUMINOID AMMONIA

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green	0.050	0.120	0.064	0.079	1.18
Mottled *A*	0.019	0.035	0.019	0.024	0.41
Mottled *B*	0.018	0.024	0.013	0.018	0.29

of Na_2CO_3 until 600 cc. of distillate was collected. An aliquot portion of it was Nesslerized and calculated as grams of nitrogen per 100 gm. of wet weight. Table XVII shows that the mottled leaves were lower in the amount of ammonia set free.

TABLE XVII

AMMONIA FROM DISTILLATION WITH Na_2CO_3

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green	0.012	0.0044	0.019	0.012	0.18
Mottled *A*	0.0073	0.0031	0.0095	0.0066	0.11
Mottled *B*	0.0056	0.0040	0.0064	0.0053	0.085

Nitrites were determined by the method as given by DAVISSON (11). Attempts were made to determine the amount of nitrite by colorimetric methods (29), but the presence of anthocyanins which could not be precipitated out with lead subacetate interfered.

TABLE XVIII

NITROGEN AS NITRITE

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green	None	None	None	None	None
Mottled	0.001,12	0.001,32	0.001,42	0.001,29	0.021,5

The fresh leaves (25 gm.) were ground finely with quartz sand, placed in the flask, and titrated as suggested by DAVISSON.

ASO and SEKINE (3) found that there was about 0.000,78 per cent N in the healthy buds of *Sagittaria sagittifolia*, when calculated on the basis of wet weight, or 7.8 parts N per million of material. In mottled *Coleus* leaves there are 12.9 parts per million, but not a detectable amount in green leaves. BONCQUET (4) reports the presence of nitrite and ammonia in various plants which were diseased. The diseases were of the physiological type such as curly leaf of sugar beets, curly dwarf of potatoes, mottled leaf of potatoes, and mosaic disease of the tobacco. He believes that

TABLE XIX

CARBOHYDRATES

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams per 100 gm. dry weight
	A. M.				
Green	0.890	0.855	1.050	0.932	13.93
Mottled *A*	0.740	0.820	0.685	0.748	12.89
Mottled *B*	1.110	1.090	0.935	1.044	16.84
	P. M.				
Green	1.435	1.370	1.430	1.412	21.08
Mottled *A*	0.780	0.850	0.825	0.822	14.17
Mottled *B*	1.020	1.040	1.155	1.073	17.30

nitrogen starvation is brought about by bacterial reduction of the nitrates to nitrites and ammonia, after the nitrates have been taken up by the roots. The response of the plant to the stimulus, he says, is manifested in biochemical, physiological, and morphological changes.

The amount of carbohydrate material in the leaves was estimated by first boiling (2.5 hours) 10 gm. of the freshly ground leaves in 100 cc. of water, to which 10 cc. of HCl (sp. gr. 1.125) was added. The sugars were then determined as dextrose by the BERTRAND (31) volumetric method. The calculations are given as grams of dextrose per 100 gm. of wet weight. Sugars were determined both in the morning and in the evening after a bright day,

to ascertain the relative amounts of photosynthetic activity in the green and mottled leaves.

The green leaves gained 0.48 gm. (as dextrose) during the day, mottled *A* gained 0.074 gm., and mottled *B* 0.029 per 100 gm. of wet weight, which shows that the photosynthetic activity was greatly reduced by mottling. In the morning the carbohydrate content of the mottled leaves in all probability consisted mainly of substances of an aplastic nature, such as the hemicellulose of the cell wall which by hydrolysis forms galactose, xylose, mannose, etc. Since the carbohydrate content probably was made up mainly of substances of an aplastic nature, one would not expect them to be exhausted by respiration or to be translocated from the leaf. WILLSTÄTTER (table XXVIII) has shown that the carbon dioxide assimilated per hour was greatly reduced as the leaves yellowed.

Water content and ash

For each determination 20 gm. of fresh leaves was used; the leaves were dried at 100° C. and then ashed at a dull red heat.

TABLE XX

DRY WEIGHT

Leaf	Sample no. I	Sample no. II	Average
Green	7.22	6.88	7.05
Mottled *A*	5.80	5.58	5.69
Mottled *B*	5.79	6.60	6.20

This decrease in dry weight is in harmony with the work of BONCQUET, or, in other words, leaves of mottled plants have a higher water content. WILLSTÄTTER (table XXVIII) showed that the water content of the leaves increased as yellowing progressed. This is due either to the fact that the materials were transported from the leaf or that respiration decreased the amount of dry matter present.

Table XXI shows that the percentage of ash when calculated on the dry weight of the leaves increased 3–7 per cent during mottling. Analyses of leaves as given by SWART (48) show that as a rule the yellow leaves gave a larger percentage of ash than

the green ones. One might construe this to mean that materials are not transported from the leaf as it yellows, for if they were transported, presumably the salts would be carried along and the ash would decrease. It seems more probable that since the assimilative activity is reduced, the respiratory products pass off as gas and so leave the salts behind. BONCQUET (4) found that plants affected with nitrogen starvation have a higher percentage of ash (when based on dry weight) than normal plants, as is shown by beets, tobacco, and potatoes.

SCHULZE and SCHÜTZ (44) have shown that the ash content of leaves of *Acer Negundo* increases as autumn approaches. The

TABLE XXI

ASH

Leaf	Sample no. I	Sample no. II	Average	Grams ash per 100 gm. dry weight
Green	1.05	0.89	0.97	13.8
Mottled *A*	0.97	0.93	0.95	16.7
Mottled *B*	1.23	1.32	1.28	20.7

TABLE XXII

ASH IN DRY SUBSTANCE

	May 7	June 6	July 5	August 2	September 3	September 25
Percentage of ash	7.23	7.52	8.06	9.17	9.43	11.29

ash of the leaves increased 4 per cent, while the ash of *Coleus* leaves increased 3–7 per cent. Ash of the plane tree leaves per unit area of leaf was shown by TUCKER and TOLLENS (49) to increase gradually until October 8, after which there was a slight decrease.

Catalase

In determinations of catalase activity only the blades of *Coleus*, exclusive of the primary veins, were used. For each determination 0.5 gm. of the leaf material was ground for 2 minutes with a little quartz sand and powdered calcium carbonate as a neutralizer. The determination was run according to the method described by

APPLEMAN (1), using 10 cc. of dioxogen as the hydrogen peroxide and suspending the plant material in 10 cc. of water. Tables XXIII and XXIV show the cubic centimeters of oxygen liberated per 0.5 gm. of fresh weight of leaves at 25° C. during 10 minutes of activity. Table XXIII shows that the catalase activity gradually

TABLE XXIII

EFFECT OF AGE OF LEAF ON CATALASE ACTIVITY

OXYGEN LIBERATED	
82.0 cc.	Top pair of leaves
88.5	Second pair of leaves
93.0	Third pair of leaves
86.0	Fourth pair of leaves
80.0	Fifth pair of leaves
74.0	Sixth pair of leaves
51.5*	Seventh pair of leaves
32.0†	Eighth pair of leaves

*Leaves still green.
†Leaves half mottled.

increased and reached a maximum in the third or fourth pair of leaves, and then decreased gradually until the leaf mottled. With mottling of the leaf it was noticed that the catalase activity dropped enormously.

From table XXIV it is evident that as the leaves mottled the catalase activity decreased greatly, even reaching a value of less

TABLE XXIV

COMPARISON OF CATALASE IN GREEN AND MOTTLED LEAVES

COMPLETELY MOTTLED	GREEN
6.3	103.0
10.0	95.0
8.0	90.0

than 1/10 that of the healthy leaves. The mottled leaves were taken from the plant 3 or 4 nodes below the green ones, and therefore age would be a factor, but it is not sufficient to account for the enormous decrease in the catalase activity.

An analysis of the leaves was made to determine the amount of nitrogen and phosphates in the F_1, F_2, and F_3 of the three types of leaves. Each morning the leaves were collected until 80 gm. was obtained for each sample; the leaves as collected were preserved in 95 per cent alcohol in ground glass stoppered, wide-mouthed bottles, and kept there until needed for analysis. An extraction

was made in 95 per cent alcohol at the temperature of the boiling solvent for 20 hours, and then for 4 hours in ether at the temperature of the boiling ether. The residue was dried, weighed, and called F_3, the ether-alcohol extract was F_1 and F_2 combined. The ether-alcohol extract was evaporated to dryness in a Freas vacuum oven at 70° C., weighed, and the portion of it which was soluble in anhydrous ether at room temperature was called F_1, and the weighed residue F_2. Aliquot parts of the three fractions were analyzed and results were calculated to grams of N or P per 100 gm. of fresh leaves.

TABLE XXV

PROPORTIONS OF VARIOUS FRACTIONS IN GREEN AND MOTTLED LEAVES, WEIGHTS OF F_1, F_2, AND F_3

Leaf	Sample no. I	Sample no. II	Sample no. III	Average
Green F_3	4.41	4.78	4.60	4.60
Green F_2	1.39	1.23	1.38	1.33
Green F_1	0.63	0.65	0.69	0.66
Total dry weight	6.43	6.66	6.67	6.59
Mottled *A*, F_3	3.66	3.94	3.74	3.78
Mottled *A*, F_2	1.61	1.46	1.68	1.58
Mottled *A*, F_1	0.16	0.13	0.10	0.13
Total dry weight	5.43	5.53	5.52	5.49
Mottled *B*, F_3	4.53	4.77	4.31	4.54
Mottled *B*, F_2	1.55	1.55	1.38	1.49
Mottled *B*, F_1	0.20	0.40	0.39	0.33
Total dry weight	6.28	6.72	6.08	6.36

In comparing the weights of F_3, it is seen that there was a decrease in weight as the leaves mottled, while the weight of F_2 increased and F_1 greatly decreased. The decrease in weight of F_3 is accounted for by the fact that the carbohydrate synthesis is considerably reduced, as was shown in table XIX, where the carbohydrates of the leaf were compared in the morning and in the evening. The increase of the weight of F_2 can be accounted for by the accumulation of salts in the older leaf. The results of F_1 showed that the sulphatides, phosphatides, nucleo-proteins, fats, etc., were greatly reduced in the mottled leaves, and many of these were nitrogen complexes.

The nitrogen in F_3 was reduced one-half or more, the nitrogen in F_2 was changed very little, while the nitrogen entirely disappeared in F_1 as the leaves mottled. This would mean that such compounds as nucleoproteins, glycoproteins, phosphoproteins, albumins, and globulins were rapidly being broken down, while the amount of derived proteins, amino acids, prolamines, ammonia compounds, and other nucleic acid metabolic products remained practically constant. Since the nitrogen in F_1 practically disappeared, it would seem that the phospholipins and amines were breaking down,

TABLE XXVI

NITROGEN OF VARIOUS FRACTIONS IN GREEN AND MOTTLED LEAVES

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of nitrogen per 100 gm. dry weight
Green F_3	0.17	0.17	0.19	0.18	3.91
Green F_2	0.018	0.017	0.021	0.019	1.43
Green F_1	0.005	0.002	0.004	0.004	0.61
Total dry weight	0.193	0.189	0.215	0.203	.....
Mottled *A*, F_3	0.06	0.06	0.08	0.07	1.86
Mottled *A*, F_2	0.017	0.018	0.027	0.021	1.33
Mottled *A*, F_1	None	None	None	None	None
Total dry weight	0.077	0.078	0.107	0.091	.....
Mottled *B*, F_3	0.05	0.05	0.05	0.05	1.10
Mottled *B*, F_2	0.015	0.010	0.006	0.010	0.67
Mottled *B*, F_1	None	None	None	None	None
Total dry weight	0.065	0.060	0.056	0.060	.....

and that the plant must be drawing upon its last sources of nitrogen before death ensues. In this connection the work of KORAPETOVA and SOBASHNIKOVA (40) is very significant. They grew seedlings of rye and barley in inadequate nutrient solutions and found that the total amount of proteins decreased as growth progressed. In mottling of *Coleus* one likewise seems to be dealing with inadequate (especially nitrogen) nutrition. Here too proteins are decomposed, probably owing to the shortage of nitrogen.

In mottled leaves the phosphates of F_3 were reduced to one-third that of the green leaves, while the phosphates in F_2 increased

about 30 per cent, and those in F_1 had almost disappeared. As the main part of the phosphorus in F_3 probably existed as phosphoprotein, there was apparently a hydrolysis of this going on to form hydrolytic products of nucleic acids. Since little or no phosphorus was found in F_1, apparently the phosphatides had nearly all disappeared. Germination (**40**) in darkness appears to be correlated with a pronounced decomposition of phosphorus containing proteins. Apparently during the mottling of *Coleus* there is a similar phenomenon. PALLADIN (**40**) showed that as

TABLE XXVII

PHOSPHATES

Leaf	Sample no. I	Sample no. II	Sample no. III	Average	Grams of phosphorus per 100 gm. dry weight
Green F_3	0.0330	0.0184	0.0213	0.0242	0.526
Green F_2	0.0013	0.0008	0.0007	0.0009	0.068
Green F_1	0.0027	0.0026	0.0030	0.0028	0.425
Total dry weight	0.0370	0.0218	0.0250	0.0279	.....
Mottled *A*, F_3	0.0069	0.0068	0.0056	0.0064	0.169
Mottled *A*, F_2	0.0018	0.0014	0.0018	0.0017	0.107
Mottled *A*, F_1	None	None	None	None	None
Total dry weight	0.0087	0.0082	0.0074	0.0081	.....
Mottled *B*, F_3	0.0067	0.0095	0.0080	0.0081	0.178
Mottled *B*, F_2	0.0018	0.0012	0.0012	0.0014	0.094
Mottled *B*, F_1	None	0.0010	0.0010	0.0007	0.212
Total dry weight	0.0085	0.0017	0.0102	0.0102	.....

proteins decomposed the inorganic phosphates increased. From table XXVII it is apparent that the phosphoproteins were being rapidly decomposed as the leaves mottled. If the phospholipins are estimated according to CZAPEK (**10**), by multiplying the amount of magnesium pyrophosphate in the ether extract by 7.27, then 0.073 per cent of the wet green leaves and 1.009 per cent of the dry weight is lecithin. In the mottled leaves the phospholipins have almost entirely disappeared. The ratio of phosphorus (table XXVII) to nitrogen (table XXVI) is about 1 to 7 in the green leaves, while in the mottled leaves the ratio is about 1 to 11.

Leaf pigments

CHLOROPHYLL *a* AND *b*.—The amount of leaf pigments of *Coleus* was compared with the amount of leaf pigments in the lilac, according to the method given by WILLSTÄTTER and STOLL (**53**). The ether extracts of the pigments were compared spectroscopically, and the amount of chlorophyll present in the lilac and in the *Coleus* was compared by the width of the absorption bands in the red end of the spectrum. In this comparison the band lying between the Fraunhofer lines *B* and *C* was used, since it was the most distinct. The extract of the *Coleus* leaves absorbed the rays from 685.5 $\mu\mu$ to 674.5 $\mu\mu$. The chlorophyll solution from the lilac leaves was then diluted until it gave an absorption band of the same width. It was found that the chlorophyll *a* and *b* content of the healthy green leaves of lilac was five times as great as the chlorophyll content of the healthy green leaves of *Coleus*. Not even a trace of absorption in the red end of the spectrum was observed in the ether extract of the completely mottled leaves.

The four leaf pigments (chlorophyll *a* and *b*, carotin, and xanthophyll) of green and completely mottled leaves of *Coleus* and of green leaves of lilac were then separated according to the method given by WILLSTÄTTER and STOLL,[1] and the extractions tested spectroscopically to make sure of the purity of each extraction. The amount of each of the four pigments in the various leaves was then compared by means of a Schreiner colorimeter. The amount of chlorophyll *a* and *b* in the lilac leaves was taken as a standard, and the amount of green pigments in the *Coleus* leaves was compared with that of the lilac leaves. The writer realizes the limits of such a standard and regrets that there is not some stable dye or color which would serve as a basis for determining the exact amount of chlorophyll pigments in a leaf. The extraction of the pure pigments is a rather lengthy and expensive process, and when the pure pigments are once obtained some of them apparently do not keep well.[2] By colorimetric comparison the lilac leaves were

[1] A method for the quantitative estimation of the four pigments of green leaves will be published later.

[2] Preparation of these pigments, their keeping qualities, and some spectrophotometric data will be published soon.

found to have about five times as much phytochlorine as the *Coleus* leaves (based on the wet weight). The same relation was found to hold for phytorhodin *g*. This means that the two leaves bear *a* and *b* chlorophyll in the same proportions, but that the lilac has about five times as much of each as the green *Coleus*. Assuming that the lilac leaves contain 0.8 per cent (the average percentage for green leaves) of chlorophyll, then *Coleus* leaves contain only 0.16 per cent of chlorophyll, based on the dry weight of the leaves.

Table XXVIII, quoted from WILLSTÄTTER, shows the relation between autumnal yellowing, chlorophyll content, and photosynthetic activity. These changes agree generally with the writer's results in the mottling of *Coleus* in mid-season.

TABLE XXVIII

CHANGES AS LEAVES YELLOW IN AUTUMN (LEAVES ALL OF SAME SPECIES)

Leaf	Date	Dry weight in gm.	Chlorophyll content	CO_2 assimilated per hour
Deep green	July 30	1.55	19.7	0.080
Green	September 17	1.55	12.5	.....
Green with yellow spots	October 5	1.45	7.8	0.064
Almost yellow	October 19	1.35	2.1	0.010

In alfalfa hay JACOBSON (**23**) has shown that 0.68 per cent of chlorophyll and 0.28 per cent of yellow coloring matter were present. The chlorophyll was estimated after the method of MARCHLEWSKI, and was shown to contain 66 per cent neochlorophyll and 34 per cent allochlorophyll. He believed that this ratio would vary, depending upon the conditions of growth.

XANTHOPHYLL.—The xanthophyll in the leaves was estimated by comparing the extracted pigment with a standard solution of potassium dichromate, as recommended by JÖRGENSEN and STILES (**26**). There was found to be present in 1000 gm. of fresh lilac leaves 0.273 gm. of xanthophyll, in the green *Coleus* leaves 0.087 gm., and in the mottled *Coleus* leaves 0.239 gm. From this it is seen that green *Coleus* has about one-third as much xanthophyll as lilac, while fully mottled *Coleus* has nearly as much as lilac. The amount of xanthophyll present in lilac was about the same as that present in *Sambucus nigra* (0.250). The amount of

xanthophyll in green *Coleus* leaves was about one-third of the amount present in most leaves.

CAROTIN.—Mottled *Coleus* leaves were found to contain about 0.3575 gm. of carotin per 1000 gm. of fresh leaves, while green *Coleus* leaves had 0.0894 gm. and green lilac leaves had 0.1324 gm. None of the leaves analyzed by WILLSTÄTTER gave as much carotin as did the mottled *Coleus* leaves. Poplar leaves (0.097) had about the same amount of carotin in them as did green *Coleus* leaves, while the leaves of *Sambucus nigra* (0.134) and *Fagus silvatica* (0.131) had about the same amount of carotin in them as did lilac leaves.

The ratio of carotin in lilac leaves was found to be $\frac{0.1324}{0.2730}$, or 0.48; for green *Coleus* leaves $\frac{0.0894}{0.0870}$, or 1.05; for mottled *Coleus* leaves $\frac{0.3575}{0.2390}$, or 1.49. WILLSTÄTTER found that the average ratio was 0.603 ± 0.1. Even in green *Coleus* leaves the carotin was higher than the average, and the ratio in the mottled leaves was greatly increased over what it should be if the leaf were normal as to its yellow pigments.[3]

Discussion of leaf pigments

Various theories have been proposed to explain how the pigments change as the leaf yellows. SWART (**48**) found that yellowing of leaves which are dying begins in that part of the parenchyma which is farthest from the vascular bundles, and takes place last in the largest vascular bundles. In connection with the disappearance of chlorophyll on aging of the leaf SWART mentioned three possibilities: the chlorophyll either was transported or it remained in the leaf when it broke down, and if it broke down in the leaf the decomposition products either were transported to the stem or else remained in the leaf. He thought that chlorophyll in the form of decomposition products passed from the leaves into the stem, while the yellow pigments remained in the leaf.

[3] For comparative work on the yellow pigments Lovibond slides have been found to be quite satisfactory.

STAHL also believes that the products of chlorophyll decomposition do not remain in the leaf, but diffuse through the veins to the stem. He gave as proof the fact that if the veins of a *Ginkgo* leaf are severed, the chlorophyll remains longer than it does in a control leaf. Similar experiments were conducted on the leaves of *Coleus* by the writer, but the leaves yellowed just the same.[4] The types of leaves are entirely unlike, hence it is not surprising that the results were different. STAHL believes further that, since the yellow pigments (carotin and xanthophyll) consist of carbohydrate materials only, they were not needed by the plant and so were left behind, but the green chlorophyll pigments which contained magnesium and nitrogen were decomposed, and these elements were carried away to meet the requirements of the plant.

SWART has shown that it is unlikely that magnesium is withdrawn from the leaf. Table V shows that the plant does not lack magnesium, and consequently there would be no occasion for it to draw upon its chlorophyll supply for the very small quantity which is present in the chlorophyll molecule. On the other hand, all the data tend to show that nitrogen is the element lacking, and therefore it is quite possible that either the chlorophyll would be prevented from forming, or if formed would be decomposed, if the law of mass action plays any part at all in the process.

The situation is summarized by MEYER (38) in the case of yellowing of *Tropaeolum* leaves. As the leaves age they become weakened. This weakening of the leaves results in curtailed assimilation, which is limited because the chloroplasts become smaller and the organs are weakened. This weakening of the organs is a primary cause, while the decomposition of the protein follows because of this. Lastly, the decomposition of the protein accelerates the yellowing of the leaf. MEYER believes that the chlorophyll decomposes and is then borne away, while the yellow pigments remain as they were in the leaf, neither increasing nor decreasing in quantity. Since he made no quantitative determinations on the pigments of the leaves, it is easy to see how he might have deduced such a conclusion in regard to the yellow

[4] On the leaves of *Ginkgo*, during the summer and autumn of 1920, at Washington, D.C., the experiments of STAHL could not be confirmed.

pigments. Had MEYER availed himself of methods of estimating the yellow pigments, he doubtless would have reported differently.

KOHL (**30**) has written extensively on carotin. He has shown that in *Vicia Faba* seedlings carotin forms and increases in the dark; in the light, at a low temperature, chlorophyll formation is suppressed, while the carotin increases; increase of light and temperature accelerates chlorophyll formation. Working with several species of plants, he concluded that etiolated plant organs owe their color almost exclusively to carotin. When some etiolated plants greened, the carotin content was found to increase sometimes as much as 125 per cent, and in all cases was found to increase to some extent. He did not believe that chlorophyll was formed at the expense of the carotin, however, nor did he think the chlorophyll was changed to carotin in autumnal coloration. His experiments on coloration led him to conclude that the carotin content (evidently including carotin and xanthophyll) of the leaves increases. His results show that the carotin content of old leaves of *Sambucus nigra* is to the carotin content of young leaves of the same plant as 183:170. Color changes which he describes are very similar to those described by MEYER in autumnal yellowing.

STOKLASA, SEBOR, and SENFT (**47**) believe that the autumnal changes of color depend on the hydrolytic fission of chlorophyll and the formation of phaeophytin and phosphatides; these substances, which themselves have a brownish color, allow the red and yellow of carotin and of xanthophyll to appear. The colorless lecithin and choline derivatives are not combined with chlorophyll, but are merely admixed and possibly intimately associated with the chlorolecithins. The writer has shown that the yellow pigments in *Coleus* greatly increase as the leaf mottles. The yellow pigments are not simply left behind when the other pigments are translocated, but are being continually formed.

WILLSTÄTTER stated that the proportion of chlorophyll *a* and *b* to the yellow pigments was 3.07 to 1 in sun leaves, while in shade leaves it was 4.68–6 to 1. IWANOWSKI (**22**) found that less chlorophyll was broken up by light when the yellow pigments were in greater concentration. He concluded that the protective action of the yellow pigments could no longer be doubted. The yellow

pigments absorb blue and especially violet rays, whose power to break down chlorophyll is especially high. It is of interest to notice how the carotin content varies under different conditions. The amount of carotin in a leaf varies according to the seasons of the year (40), being greatest during the flowering period in nettles and horse chestnuts. In vetch (40) about five times as much carotin exists in the green leaves as in the etiolated ones.

EWART (16) has shown that when mustard seedlings were grown in the absence of carbon dioxide, more carotin was produced than when carbon dioxide was present. He remarks that his method of analysis was not wholly accurate, however, and that during the separation of the pigments the losses were so great that the exact estimation of the amounts originally present was impossible. The seedlings grown in air deprived of carbon dioxide were

TABLE XXIX

AMOUNTS OF PIGMENTS PRODUCED IN MUSTARD SEEDLINGS (PER 100 GM. OF FRESH MATERIAL)

CO_2	Chlorophyll	Carotin	Xanthophyll
Present	0.474	0.11	0.15
Absent	0.271	0.275	0.12

smaller, darker, and more bluish green. This blue green seemed to be due not to an excess of chlorophyll, but rather to the more compact character of the tissues, and it appears that chlorophyll develops most rapidly when its normal functional activity can be exercised. EWART believes that he is justified in concluding that the carotin supplied at least a part of the carbon and hydrogen for the construction of chlorophyll.

KOHL (30) showed that etiolated seedlings contained carotin in abundance, and even doubted whether any other pigment was present. He found that the percentage of carotin did not decrease when the etiolated seedlings were exposed to light and chlorophyll was formed. He thus denied that carotin was converted into chlorophyll as was believed by EWART. EWART states that while food materials were abundant the production of carotin continued at a greater rate than it was used in the formation of chlorophyll.

He showed that etiolated wheat seedlings contained 8–10 parts of carotin to one of xanthophyll, and that leaves of *Hordeum murinum* when kept in darkness turned yellow or yellowish red. When analyzed for plant pigments, the leaves were found to contain chlorophyll, xanthophyll, and carotin in the proportions 1, 3, and 12, respectively, and in addition a red pigment, which was apparently a flavone, believed to be a decomposition product of chlorophyll. His suggested explanation of the greening of etiolated plants is unique. When an etiolated plant turns green in light, the carotin undergoes photo-oxidation. The bleached carotin residue combines with glaucophyllin, converting it into the tricarboxylic chlorophyll.

WILLSTÄTTER found that a weak alcoholic oxalic acid solution splits (in the cold) magnesium out of the chlorophyll molecule. On this basis SWART assumes that the decomposition of chlorophyll in yellowing leaves is due to acids, thus splitting the chlorophyll molecule. This postulates an increase of acidity with yellowing, a theory for which there is no evidence. SAMPSON (**43**), in testing the acidity of *Coleus* leaves, found that fresh yellow leaves in the act of abscissing had an acidity equivalent to 0.0069 cc. of N acid per gram of wet weight, while fresh green leaves had an acidity equivalent to 0.0089 cc. of N acid. Since the green leaves are more acid (at least as measured by their base absorbing power) than the yellow ones, if SWART's assumption is correct, one would expect to see the top leaves of the plant yellow instead of green. It is hardly probable that the splitting of magnesium out of the chlorophyll molecule, due to acid accumulation, is the first step in chlorophyll decomposition. PALLADIN (**40**) pointed out that carbohydrates are essential to the formation of chlorophyll. From the sand culture experiments with *Coleus* one could hardly say that the deficiency in carbohydrates caused the chlorophyll to disappear. It would be more accurate to say that the carbohydrate output was decreased, owing to the deficiency in chlorophyll.

WIESNER (**52**) supposed that the chlorophyll in the living leaf was dissolved in an oil, in which the concentration of chlorophyll was very high and the decomposition very low. IWANOWSKI (**21**) agreed with WIESNER in regard to the concentration of the chloro-

phyll, and has proved that plants with much chlorophyll show little or no breaking up of chlorophyll by light, and that plants with little chlorophyll (as *Elodea*) show as much as 31 per cent of the chlorophyll broken up by light in seven hours. IWANOWSKI also showed that colloidal solutions were about sixteen times as light stable as molecular solutions, and the more concentrated the colloidal solution the more light stable it becomes. HERLITZKA (**20**) also found that the chlorophyll exists in the leaf in the colloidal state.

In view of these facts one might expect light lability to enter as a factor in the decomposition of chlorophyll in *Coleus*, for the chlorophyll is only one-fifth as concentrated as it is in most other sun plants.

BORESCH (**6**) in his experiments on algae (*Phormidium corium*) showed that the algae when grown on nutrient media changed from a dark green, after two months, to gold or red brown. Addition of more of the nutrient media caused them to resume again their green color. He believes that the change of color back to the natural green was due to the presence of the nitrogen in the potassium nitrate. Any nitrate, ammonium salt, or other nitrogen compound would do the same. Other plants, such as *Chlamydomonas*, *Hydrodictyon*, and *Oedogonium*, depend upon nitrogen compounds for their existence, and also the building and accumulation of chlorophyll depends upon the available nitrogen supply. Extractions of the pigments were made by BORESCH, who found that the green Cyanophyceae showed the normal colors (chlorophyll, phycocyan, and carotin), while the brownish extracts of these algae showed little chlorophyll and much of the carotin. He believes that the brown color was due to the breaking down of the chlorophyll and phycocyan, which are closely related in their origin. He also noted that the carotin increased as the chlorophyll and phycocyan broke down. In the case of higher plants, BORESCH noticed that nitrogen had a greening effect upon the plants. When nitrogen was failing and the leaves were getting yellow, additions of manure kept them green. ARTARI (**2**) found that chlorophyll formation and the quantity of chlorophyll depend upon the substratum. *Stichococcus bacillaris*, when grown in the dark with

nitrogen sources such as asparagin, peptone, and ammonium nitrate, greened, and when potassium nitrate was used it became pale or colorless. If *Stichococcus* were grown in the light and fed upon rich organic nutrients (maltose, glucose), it lost its chlorophyll. If the colorless algae which had been growing in the dark were placed in the light and given potassium nitrate as nutrient, they regained their normal color and the chromatophores became normal again. He did not know whether the chromatophores were built up anew or not. ARTARI also found that when he placed the colorless algal cells on a nutrient solution which contained either asparagin or ammonium nitrate, and placed them in the dark, the algae greened again.

TABLE XXX

COMPARISON OF GREEN AND YELLOW PIGMENTS IN LEAVES OF HEMP PLANTS UNDER DIFFERENT TREATMENTS

Process	Chlorophyll	Carotin
Intense manuring	100	100
Complete manuring	74	90
Nutrients lacking nitrogen	38	57
Nutrients lacking phosphates	71	80
Nutrients lacking potassium	66	72
Nutrients lacking calcium	72	90
No nutrients added	53	71

The effect of the various nutrient elements is perhaps best described by VILLE (51). He experimented upon the effect produced on the color of many field plants by a deficiency in nitrogen, calcium, phosphate or potassium, and no manuring. He found that nitrogen affects chiefly the color of the plants, and if it is deficient the plants become brown, while if the dose of nitrogen is increased or diminished, the color increases or diminishes accordingly. He made crude extracts of the leaves of hemp and compared the green and yellow pigments in them. Table XXX shows the results of VILLE's experiments.

The *Coleus* here studied seems to be on the verge of nitrogen starvation at all times under ordinary greenhouse conditions. It is only with considerable nitrate additions that the nitrogen supply of the leaf can be maintained sufficiently to avoid chlorophyll

decomposition. It is a great nitrogen user, and it is difficult to know what use is made of all the nitrates it consumes. One wonders whether there is a denitrifying process going on within the leaves which keeps the nitrates more or less depleted, and in case of lack of continuous additions completely removes them. This or some draft on the nitrogen of the leaf leads to a decomposition of nitrogen compounds of the leaf, including proteins, phospholipines, and chlorophyll, the decomposition of the latter producing the mottling. All the experimental evidence points to the shortage of nitrogen as the cause of chlorophyll decomposition in the plant studied, a plant very prone to mottling. One would hardly expect the same limiting factor to determine chlorophyll decomposition in plant organs in all cases, but many of the cases of chlorophyll loss discussed, whether involving loss from plants grown in cultural solutions, or in soils in the midst of the growing season, or loss accompanying autumnal coloration, point in the same direction. In the *Coleus* studied it is evident that there is little ground for SWART'S view that high acidity of the leaves leads to a decomposition of chlorophyll by splitting magnesium out of the molecule. Cultures with cuttings of this variety of *Coleus* also show that under ordinary cultural conditions the plant bears little margin of phosphate supply, while it bears a great excess of calcium, magnesium, and iron. The narrow margin of phosphate supply does not manifest itself in mottling, but only in limited growth. The narrow margin of nitrogen, on the other hand, manifests itself both in limited growth and in mottling. If IWANOWSKI is correct in his assertion that carotin and xanthophyll render chlorophyll more nearly light stable, an increase in these pigments during mottling may act in a protective way against decomposition of chlorophyll.

From the various investigations presented two things are of striking interest: the decomposition of chlorophyll and the great amounts of yellow pigments which are present when chlorophyll is absent, whether it is in etiolated plants, algae grown in the dark, plants which have poor nutrition, or when leaves mottle. Since the carotin is present in greater amounts when chlorophyll is absent, and since carotin apparently decreases as chlorophyll

increases, one would naturally seek the cause of this, and is led to investigate the relationships which may exist between the various plant pigments.

EWART (**16, 17**) believes that carotin after photo-oxidation or partial disintegration forms a massive hydrocarbon combination (the phytol radicle of chlorophyll) whose addition is necessary to convert the dicarboxylic glaucophyllin into the tricarboxylic chlorophyll. Perhaps this might explain the disappearance of carotin as chlorophyll forms. The bridge between the two yellow pigments is not so difficult to cross, for one of them (xanthophyll) is simply the oxidation product of the other (carotin).

Some believe that chlorophyll may act as a sensitizer, and others that it may act as a photic or lytase enzyme which converts carbon dioxide and water into carbohydrates. EWART (**16**) has shown that chlorophyll may act as an enzyme according to the following three equations:

(1) $2\ C_{31}H_{29}N_4Mg(COOH)(COOCH_3)(COOC_{20}H_{39}) + 36CO_2 + 16H_2O = 2\ C_{40}H_{56}O_2 + 44O_2 + 2C_{31}H_{30}N_4Mg(COOH)_2$ Amorphous chlorophyll+carbon dioxide and water would form xanthophyll or carotin, oxygen, and glaucophyllin. (2) $C_{40}H_{56}O_2 + 24H_2O + 7O_2 = 2\ C_{20}H_{39}OH + 3C_6H_{12}O_6 + 3C_6H_{12}O_6 + 4$ HCHO. Carotin or xanthophyll+water+oxygen equals phytol+levulose+glucose+formaldehyde. (3) $2C_{20}H_{39}OH + 2C_{31}H_{30}N_4Mg(COOH)_2 + 4CO_2 = 2C_{31}H_{29}N_4Mg(COOH)(COOCH_3)(COOC_{20}H_{39}) + 3O_2$. Phytol+glaucophyllin and carbon dioxide form amorphous chlorophyll and oxygen.

Reaction 2 takes place in the light with the aid of an oxidase enzyme.

Since many chemical reactions are reversible, and since phytol splits off the chlorophyll molecule easily, it is possible that the sugars and phytol may react to form carotin or xanthophyll, which would account for the greater accumulation of yellow pigments in autumn leaves and also for the disappearance of the chlorophyll. Film experiments carried out by EWART (**17**) showed that carbon dioxide combines with chlorophyll to form xanthophyll and a colorless waxy substance. The combination takes place in the presence of water and is accelerated by sunlight. It is thus seen

how the yellow pigments in the leaf may increase at the expense of the chlorophyll.

Bacteria

The leaves of *Coleus* were examined closely for the presence of bacteria. In the healthy green leaves a few bacteria of the coccus type were observed, while in the fully mottled leaves many bacteria of this type were found. A few of the bacillus type were also present in the cells. The presence of ammonia and nitrite can possibly be accounted for by their activity. Plates were made of the leaves under sterile conditions so as to get only those bacteria which were inside the cells, and always a much greater bacterial count was obtained from the mottled leaves. It is realized that the bacterial side of this question is really a problem in itself, and that this phase of the subject ought to be further investigated.

The juice of the mottled leaves was placed on the under side of the healthy green leaves and rubbed around; in other cases, in addition to rubbing the juice on the leaf, the veins were injured mechanically. The leaves in these cases mottled no sooner than did the leaves of the untreated plants, and they mottled in exactly the same manner as untreated plants. The writer believes that the bacteria get a better hold as the leaf weakens from nitrogen starvation. Evidently the organism present is a denitrifying one, which develops somewhat in the green leaf, and as the leaf weakens or mottles the organism develops more rapidly.

It will be worth while to summarize what other workers have discovered about certain bacterial and physiological diseases which in some respects appear to be similar to the mottling of *Coleus*. FREIBERG (**18**) inoculated varieties of pumpkins, squash, watermelon, cucumber, citron, muskmelon, and others, and not a single infection resulted from his inoculations, yet during the same season other plants of these same varieties contracted the mosaic disease. JAGGER (**24**) and DOOLITTLE (**13**) report that the mosaic leaf disease of cucumber is transmissible by rubbing the healthy plants with crushed diseased leaves, and have proved that *Aphis Gossypii* transmits the mottled leaf disease of the cucumber. SMITH and BONCQUET (**45**) state that *Eutettix tenella* is the only carrier

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of the disease-producing agent of the curly leaf of sugar beet. STEWART and REDDICK (**46**) report that the mosaic disease of beans is transmitted through the seeds, and that healthy seedlings rubbed with crushed diseased leaves showed infection four weeks later. MCCLINTOCK (**36**) noticed that several varieties of lima beans mottle, while the larger variety growing with these does not mottle. He thought that the bean mosaic disease was carried by the seed. EDSON and SCHREINER (**15**) state that ORTON observed a potato disease characterized by bronzing and later browning of the leaf. This disease appeared in New Jersey, but was absent in areas which were treated with potash or stable manure. They believed that the primary cause was insufficient potash or perhaps an excess of nitrates in the presence of a minimum potash supply.

BONCQUET (**5**) found that the mottled leaf of beets, tobacco leaves, mottled potato leaves, and many other mottled leaves were associated with bacteria. The normal green leaves of these plants gave no nitrite or ammonia tests, while usually the mottled leaves showed the presence of nitrites and free ammonia. The fact that a leaf is yellow, however, is no sign that nitrite or ammonia is present in it. BONCQUET thought that the mottling of the leaves was a pathological disturbance brought about by the partial and local nitrogen starvation of the tissues. Nitrogen starvation in the leaves around nitrate-reducing foci explained the mosaic nature of the leaf diseases in which an abundance of nitrite was detected. Potato plants growing in soil rich in nitrate may yellow and mottle, owing to the presence of nitrate-reducing bacteria in abundance.

Summary

1. The leaves of *Coleus Blumei* (var. Golden Bedder) are very prone to mottling or loss of chlorophyll. Mottling progresses from the lower leaves upward. Mottling of the leaves takes place first at the edge of the leaf and progresses slowly toward the veins and to the base of the leaf. Usually the pair of leaves immediately above these mottles next, and so on as the plant grows.

2. In mottling the chloroplasts lose their green color, become reduced in size, and carry on very little photosynthesis.

3. In the usual greenhouse cultural conditions, this plant has within it phosphorus and nitrogen little in excess of its immediate needs. This was shown by cuttings grown in phosphate-free nutrient mixtures; failure to grow was noticeable, although the plant maintained a healthy green color. When the plants were transferred to nitrate-free mixtures, they failed to grow and also lost their natural green color.

4. Under usual cultural conditions these plants seemed to have within their tissue magnesium, calcium, and iron greatly in excess of their immediate needs. Cuttings grown in nutrient mixtures lacking any one of these elements grew and maintained their normal green quite as well as in the complete nutrient mixtures.

5. A deficiency of magnesium or calcium apparently has nothing to do with mottling.

6. More iron was found in all parts of the mottled than in the green leaf.

7. A deficiency in phosphorus caused a larger percentage of the leaves to drop than did a deficiency in iron, magnesium, calcium, or nitrate.

8. A deficiency in phosphorus caused more of the leaves to drop, while a surplus of phosphorus did not prevent them from falling if nitrogen was deficient.

9. The effect of adding nitrogen to a plant or withholding it was shown in a very few days by the change in color of the leaves.

10. Addition of a nitrogen compound (sodium nitrate) to a plant potted in soil kept the leaves on and the plant green, while the addition of iron, magnesium, calcium, or phosphate made very little change in the appearance of the plant.

11. In order to maintain a healthy condition and a green color the plants seemed to require more nitrate than other plants of which we have a record.

12. The mottled leaves always had a lower percentage of nitrate nitrogen, protein nitrogen, ammonium salts, and albuminoid ammonia than did the green leaves; mottled leaves had nitrites and free ammonia present in them.

13. The general appearance of the mottling was the same as that of the leaf of citrus fruit trees.

14. Mottling of the leaves greatly lessened the carbohydrate output.

15. The catalase activity of the leaves was very greatly reduced as the leaves mottled.

16. The dry weight of the mottled leaves was less and the ash greater than that of healthy green leaves.

17. In mottled leaves the weight of F_1 and of F_3 was less, while that of F_2 was more than that of the green leaves.

18. Protein nitrogen decreased in all of the three fractions as the leaves mottled, while phosphates decreased in F_3, increased in F_2, and almost disappeared in F_1.

19. The amount of chlorophyll (*a* and *b*) was about one-fifth of that of lilac leaves; the proportion of *a* to *b* was the same as in other plants (lilac).

20. The carotin and xanthophyll content greatly increased as the leaves mottled.

21. Bacteria were found within the cells of the mottled leaves, but it is not known whether they bear a causal relation to mottling or not.

Great indebtedness is due to Professor WILLIAM CROCKER for his keen analytical interest and advice, and to Dr. S. H. ECKERSON for her untiring interest and suggestions on the problem.

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GAMETOPHYTIC DEVELOPMENT OF BLISTER RUSTS[1]

J. F. ADAMS

(WITH FOUR FIGURES)

Since the discovery of the pycnial stages for the stem forms of blister rusts on pines, several interesting points have arisen with respect to their alternation with the aecial stage. The pycnial stage of rusts on Angiosperms usually precedes the appearance of the subsequent stage (aecia, primary uredinia, or telia) by a few days to several weeks. The interval of time in completing the gametophytic development in the case of the stem rusts on pines is materially different.

WEIR and HUBERT (9) have added much to our knowledge regarding the appearance of pycnia of several blister rusts. Pycnia of *Cronartium cerebrum* on *Pinus Banksiana* were collected as early as May 12, 1916. Four of the galls developed aecia within 4 or 5 mm. from the pycnial exudations on the same galls, but not from identical pycnial areas. The pycniospores of *C. Comptoniae* developed similarly to those of *C. Comandrae* in respect to their appearance on previously unruptured tissue. They secured exudation of pycniospores by laboratory forcing methods from specimens collected on May 12, 1916, at Cass Lake, Minnesota. They report as follows:

> In *Cronartium coleosporoides* the pycniospores are produced on old galls previously ruptured, as well as on unruptured infected tissues . . . . In a period from April 4 to 15, 1916, abundant pycnial exudations containing pycniospores were obtained from galls of *C. coleosporoides* . . . . The pycniospores of *C. Comandrae* apparently develop but once on the same tissue preceding the appearance of the aeciospores. The production of aecia kills the infected tissues which are included in the aecial ruptures. The tissues bordering this area are invaded by the mycelium of the fungus, produce swellings, and give rise to pycniospores, either in early spring or in late summer and fall, whenever sufficient time has elapsed from the last production of aecia. In the cases recorded the pycniospores appeared in the same season following the production of aecia, with only five months intervening, but not from the identical area from which the pycnia were produced.

[1] Contribution from the Department of Botany, The Pennsylvania State College, no. 27.

BOYCE (2) first observed the pycnia of *Peridermium pyriforme* on *Pinus ponderosa* near Castella, Shasta County, California, July 21, 1916. He states that "the pines are probably infected in the summer or fall of one season, pycnia do not appear until the summer of the next season at the earliest, while mature aecia are produced in the late spring or early summer of the third season."

HEDGCOCK, BETHEL, and HUNT (6) state that the pycnia of *Peridermium pyriforme* are borne on areas of the bark of pines contiguous to the aecia and preceding them by one year. They are produced in the portions most recently invaded, commonly on the trunks and limbs. In both *P. pyriforme* and *P. filamentosum* the pycnia most frequently appear at a date later in the season than the aecia.

HEDGCOCK and LONG (7), from field observations during a period of four years, find that in the swellings of *Peridermium cerebrum* or *Pinus virginiana* the pycnia precede the aecia one year, instead of preceding them during the same spring. In other words, the pycnia and aecia occur during alternate years, and two years is the time required for a life cycle of all forms of spores of the rusts.

DODGE and ADAMS (5) studied material of *P. cerebrum* on *Pinus rigida* and *P. virginiana*, and our observations indicate that there is an alternation of aecia and pycnia.

> We have not seen in any instance spermatial hyphae developing in the tissue overlying that in which the aecidia are being formed. Cross-sections of the Virginia material developing both spermatial and aecidial fructifications on the same gall show that there is no sharp line of demarcation between the two. In one burl there was a space of only 700 μ separating them.

SHIRAI (8) has shown by culture experiments the connection of *Peridermium giganteum* and *Cronartium quercuum*, which has been considered the same as the form of *P. cerebrum* in North America. In an illustration he shows the extended pycnial layer in the tissue overlying the aecia, and states:

> The spermagonia of this fungus are formed in the month of January in the intercellular spaces between the corky bark and the corticial parenchyma. . . . . In consequence of the formation of the spermagonia and the subsequent cracking of the corky bark, the pressure of the latter on the inner bark greatly lessens, and thus secures the formation of the aecidial layers in the deeper tissues.

I have examined specimens of *Peridermium Strobi* collected May 5 and 21, 1917, in New Hampshire by Dr. L. O. OVERHOLTS. The specimens were in sporulating condition. In every instance the mature pycnial layer was found intact in the tissue overlying the aecia. COLLEY also states that "the pycnia of *Cronartium ribicola* precede the aecia by at least one growing season on any given area of infection, and succeeding generations of pycnia and aecia follow a more or less definite schedule." In this case the perennial infection is not restricted, as found with some other stem rusts. It is a typical progressive infection. The development of pycnia and aecia of the leaf rusts of conifers apparently is correlated with the time of infection. In the case of *Peridermium acicolum* mature spermagonia have been found as early as March 1, and the aecial primordia developing May 25, 1917, only an interval of about three months intervening between the appearance of pycnia and aecia. The infection of the needles occurs the preceding fall. Mature aecia of *Peridermium Peckii* were collected May 20, 1917, at Pine Grove Mills, Center County, Pennsylvania. The pycnia precede the aecia by two to three weeks, according to field observations. The same condition occurs in *Coeoma Abietis-canadensis*, which is found on the cones and new terminal growth of *Tsuga canadensis*. The infection with these two species occurs the same spring. These observations indicate that with respect to the seasonal interval there are at least three methods in the sequence of pycnia and aecia (or the completion of the gametophytic development on any given infected area).

1. The first method is the alternation of pycnia and aecia in *Peridermium cerebrum*, as reported by HEDGCOCK and LONG (7), and DODGE and ADAMS (5). The evidence supports the contention that it takes two years to complete the gametophytic period of development on any given infected area. The pycnia appear in spring and are sloughed off about the middle or end of the same growing season. The following spring the aecia are developed. It is not until the third season that the pycnia again are developed. This condition may occur on the same gall, in which case apparently one part is an older infection. It is usually found that such areas represent different stages of maturity, and are differentiated by furrows.

Whether the form of *Peridermium cerebrum* is the same as *P. giganteum* remains to be established. SHIRAI describes as well as illustrates the pycnia in the tissue overlying the aecia, which would not agree with our observations on the American form. It is possible that environmental conditions may be concerned in this instance.

2. The second method is that in which maturity of the pycnia precedes the aecia in adjacent as well as the overlying tissue within a period of about six months. The full cycle of development of pycnia and aecia on the coniferous host in the same area is completed within a period of twelve months. In contrast with the first method the pycnia and aecia are sloughed off at the same time. The writer (1) has shown that the pycnia occur in the tissue overlying the aecia in *Peridermium Comptoniae* and *P. pyriforme*. Being sloughed off at the same time indicates there is no alternation as found with *P. cerebrum*. It was not known when the pycnia developed, since the exudation of pycniospores was never observed in the field when this material was collected. The presence of the pycnial layer, however, could be recognized by carefully removing the bark. At this time the pycnia appeared as an extensive olive green layer, irregular in outline, and mature. On October 17, 1919, in the vicinity of State College several infections of *Peridermium Comptoniae* on *Pinus virginiana* were observed with exudations of pycniospores. Their appearance was similar to the description given by WEIR and HUBERT. On removing the bark the pycnial layer was lemon yellow in color. Cross-sections showed the usual extensive crustlike layer of the pycnia. There were extensive pycnial primordia which indicate the immature development of the pycnial layer. From these observations and others it would appear that *P. Comptoniae*, *P. pyriforme*, *P. coleosporoides*, and *P. Strobi* represent a group of species in which the pycnia and aecia complete their period of development within twelve months. No doubt certain environments or other conditions may alternate the period of time the pycnia may appear in one season, but it seems probable that this sequence is more or less regular after the first period of development has been completed. The completion of the initial gametophytic period of development

appears to be variable. CLINTON (3, 4) has shown, by infection of white pines with telia of *Cronartium ribicola,* that the pycnia may appear with the initial infection after a period of six months, one year, two years, and sometimes three years.

3. The third method is found with the leaf rusts of conifers when the period of development is completed within one growing season. This is similar to the period of pycnial and aecial develop-

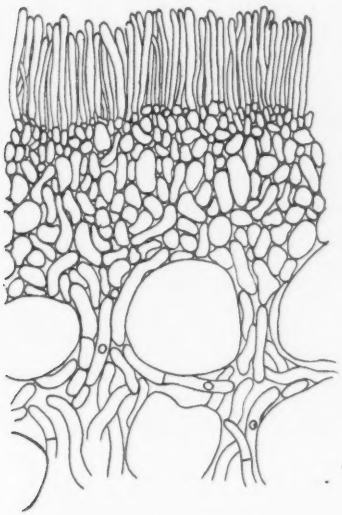

FIG. 1

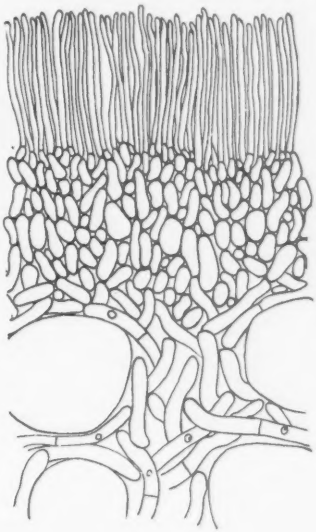

FIG. 2

FIGS. 1, 2.—Fig. 1, cross-section of pycnial layer of *Peridermium Comptoniae* on *Pinus virginiana;* fig. 2, cross-section of pycnial layer of *Peridermium cerebrum* on *Pinus rigida;* drawn with camera lucida at same magnification.

ment of Angiosperms. I have found mature pycnia of *Peridermium acicolum* March 1, 1917, at Pine Grove Mills, Center County, Pennsylvania, while mature aecia were collected May 25, 1917. In the case of *P. Peckii* the pycnia were observed to precede the aecia by only two or three weeks; this difference may be correlated with the time of infection. With *P. acicolum* infection occurs in the fall; while with *P. Peckii* infection occurs after the new growth is developed in the spring.

The first method is in striking contrast with the usual pycnial and aecial relation of rusts on the Angiosperms; the second method appears to be intermediate; while the third method apparently is the one most prevalent with all species of rust. Perennial infection on the Angiosperms is found to complete the gametophytic development in one season, regardless of being heteroecious or autoecious.

Much confusion arises as to the determination of *Peridermium cerebrum* and *P. Comptoniae* on certain species of pines, owing to

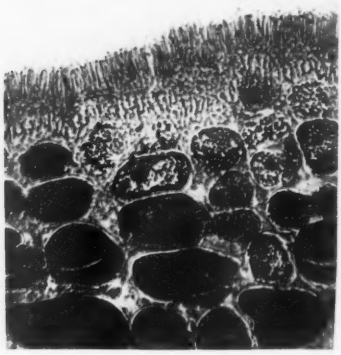

FIG. 3

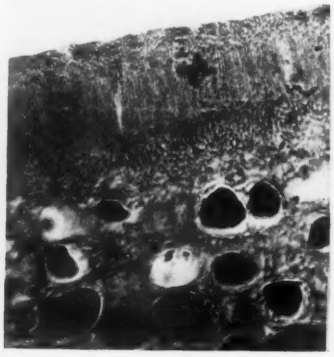

FIG. 4

FIGS. 3, 4.—Fig. 3, cross-section of pycnial layer of *Peridermium Comptoniae* on *Pinus virginiana;* fig. 4, cross-section of pycnial layer of *Peridermium cerebrum* on *Pinus rigida;* photomicrographs taken at same magnifications.

the similarity of infection upon the host. The types of infection are represented by fusiform, globoid, and semigloboid swellings. While the peridium and dehiscence of the aecia are good characters for differentiating these two species, it often occurs that specimens are immature or past their maturity for these characters to be depended upon. Under such conditions material collected in the spring or late summer may be differentiated by the pycnial characters. If the aecia are mature one can examine for the presence of pycnia in the overlying tissue. Providing pycnia are found, the form would agree with *P. Comptoniae;* if pycnia were absent, the form would agree with *P. cerebrum.* Exudation of pycniospores

does not have to be depended upon. The presence of pycnia is best determined by carefully removing the overlying bark from the cortex. The pycnia develop in the subcorticular tissue. Free-hand sections can easily be cut and mounted with a little dilute alcoholic eosin which provides a satisfactory means for examination. The pycniophores of *P. Comptoniae* are shorter and more uniform in diameter throughout their length than those of *P. cerebrum* (figs. 1–4). It is found that the pycniophores are longer and more tapering with *P. cerebrum*. No conspicuous difference in size could be found between the pycniospores of the two species. The following measurements for comparison are taken from killed material and stained with Fleming's Triple. The length of the pycniophores was taken from the sub-basal cell to the tip, and for *Peridermium Comptoniae* was 15–27 μ; while for *Peridermium cerebrum* it was 30–36 μ. A comparison of the pycniophores in these two species is shown in figs. 1 and 3, drawn at the same magnification.

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DEVELOPMENT OF HEAD AND FLOWER OF DIPSACUS SYLVESTRIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 278

HILARY S. JURICA

(WITH FOURTEEN FIGURES)

Introduction

Epigyny and its occurrence among the upper Sympetalae have frequently been studied, but epigyny begins with the Rubiales, and while almost every family in this region has received attention in this regard, the Dipsaceae have been overlooked altogether. Accordingly this investigation was begun in an endeavor to fill this vacancy. During the course of the study, however, the development of the head was found to be interesting, and since it raised the question as to the relation the developing head bears to the general topography of the plant, it was thought best to include both of these phases in the present work.

For the material used in this study I am indebted to the generosity of CHARLES C. DEAM, State Forester of Indiana, who not only collected the necessary plants at repeated intervals, but also loaned a number of mounted specimens from his herbarium. The fresh material was killed in a stock solution of chromoacetic acid and stained for the greater part with safranin, Delafield's haematoxylin, and orange G, the latter stain being omitted in a few cases.

My acknowledgment is due to Professor CHARLES J. CHAMBERLAIN, at whose suggestion the work was undertaken, for valuable assistance rendered during the course of the investigation.

General topography

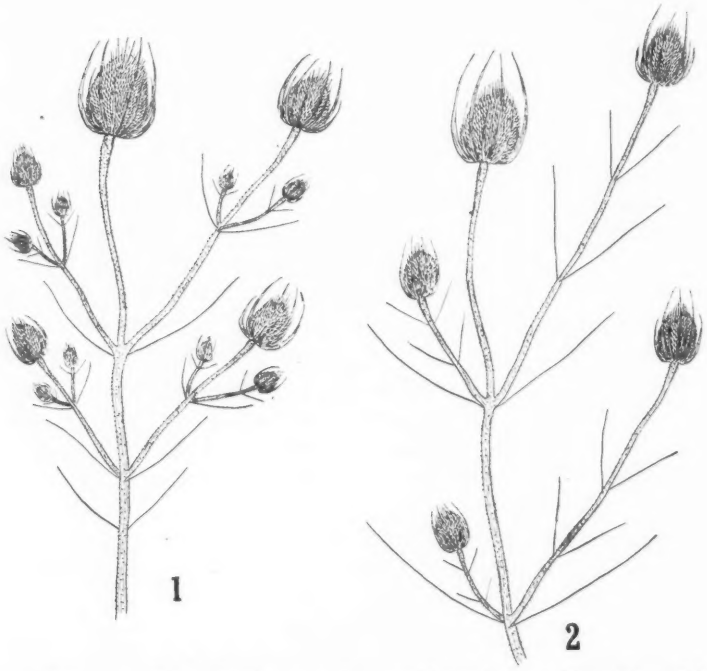
Dipsacus sylvestris is a biennial propagated by seeds. During the first season the plant develops a very flat rosette of crinkled leaves (2). These are oblong to lance-shaped, obtuse, tapering at the base and scalloped toothed. The surface of the wrinkled

leaves is deep green, while the veins and midribs are nearly white and beset with spines. A stout taproot, often more than a foot long and with many branching rootlets, serves to anchor the plant. During the second season the flat rosette sends out a shoot, which develops into a stout, erect, and strongly ridged stem, beset with sharp spines (**3**), which often grows to a height of 3–6 ft., the degree of branching depending upon the richness of the soil. If viewed in cross-section, the stem is rather tough and woody, characterized by a large pith, which gradually breaks down, so that the older portions of the stem are hollow. No attempt has been made to include a study of the anatomical features. The stem leaves occur in pairs, opposite each other, with their bases so closely clasping the stem that often they are united at the base, and thus form cups which retain water (**3**). The clasping leaves do not make their appearance on the stem in the same plane, but observe some degree of regular alternation. Thus, if one pair of leaves is pointing north and south, the alternating pairs (the next pairs immediately above and below) point east and west (**2**).

According to VELENOVSKY (**8**), the axils of leaves as a rule contain an active meristem, which may give rise to new branches or axillary shoots. This is precisely what takes place in *D. sylvestris*, although with some modification. New branches or shoots do not develop from the axils of all clasping leaves, for some abortion takes place, especially in the case of plants growing in poor rocky soil. In such an environment the plants of this species are branched very little, and in some cases not at all. Whenever new shoots or branches are given off, however, they always occur in the axils of the clasping leaves. If two or more succeeding pairs of clasping leaves give rise to lateral branches, as is often the case with *D. sylvestris* found growing in a slightly better soil, the shoots make their appearance at right angles to the ones above and below. While the lateral branches are still small and scarcely noticeable, except upon closer examination, in turn they also give rise to new daughter shoots from the axils of their clasping leaves, which follow the same general plan of development as those arising from the main or central stem. Plants with secondary branches are common, whereas plants with tertiary or quarternary branching are less

frequent, being met with generally only in more favorable environments (figs. 1, 2).

The occurrence of clasping leaves on a lateral branch has considerable uniformity, at least for the individual plant. Ordinarily a lateral branch has but one pair of clasping leaves, and its



FIGS. 1, 2.—Fig. 1, showing general topography and mode of branching, with single pair of clasping leaves per branch; positions of leaves indicated by lines; fig. 2, plant with two pairs of clasping leaves per branch, secondary branching being absent altogether.

daughter branches likewise have but a single pair; that is, the secondary, tertiary, etc., branches, each have but one pair of clasping leaves. Plants having two pairs of clasping leaves on every branch are also characterized by a similar uniformity, consisting of the total absence of further or secondary branching

(fig. 2). Probably the presence of an additional pair of clasping leaves per lateral branch is responsible for the total absence of secondary branching, especially since all the specimens examined showed this regularity. For the sake of certainty in this regard, however, a more extensive field study is necessary. Another noteworthy feature in regard to the development of new branches is the inequality of a developing pair. At times this inequality is so marked that a given shoot or branch happens to be three or even five times as long as its immediate neighbor, arising from the axil of the leaf directly opposite. This undoubtedly is due to the fact that the development of both members of a pair of lateral branches is not simultaneous.

Floral head

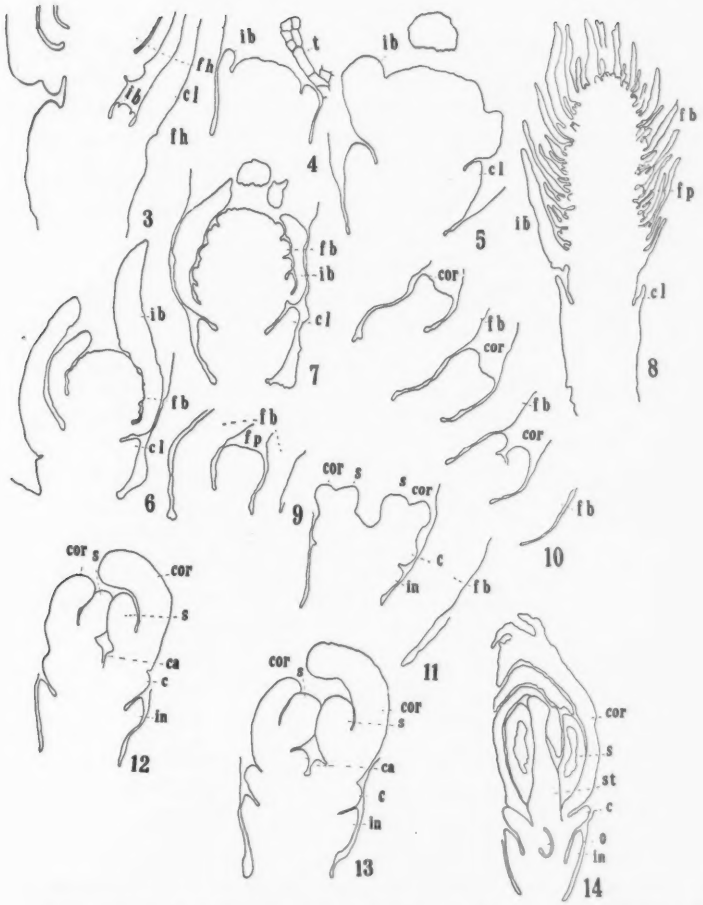
The type of inflorescence of *D. sylvestris* is a head or capitulum, surrounded by an involucre of long upcurving spiny bracts arranged in cycles of five, with the members of the outer cycle extending beyond the head, which they encircle. The bracts forming the succeeding inner cycles gradually become shorter, so much so that the members comprising the fifth inner cycle are almost equivalent in length to that of the bracts subtending the individual flowers of the head.

In its development the floral head is intimately associated with the origin of lateral or secondary branches, for the central or main stem, as well as every individual branch, is terminated by this type of inflorescence. The minute protuberance arising from the axil of a clasping leaf, and destined to develop into a new branch, is in reality a capitulum initial. Insignificant though it is at first, it immediately begins to round out, and the bracts which enter into the composition of the involucre are prompt in making their appearance (fig. 4). Almost concomitant, or at least following in close succession, are the clasping leaves of the new shoot, which appear as lateral outgrowths or papillae just below the origin of the bracts (fig. 5). As soon as the initials of these members are differentiated, the entire new shoot becomes one mass of growth. Not only does a rapid elongation of the region, both directly above and below the origin of the leaf initials, set in, but this elongation

itself has hardly had an opportunity to proceed at all, nor have the clasping leaves developed to any extent, when in their axils an active meristem begins to give rise to the initials of another floral head. By this time the outermost cycle of bracts forming the involucre has fairly grown beyond the floral head, which they encircle, and the entire capitulum becomes one mass of protuberances, the initials of the bracts, which later subtend individual flowers.

Flowers

The flowers of *D. sylvestris* are arranged in the capitulum in the form of a low spiral, and so appear to be set in diagonal rows (figs. 3–14). The method of blossoming is unique. Ordinarily one would expect the blossoming to begin at the base and extend toward the tip, but the blossom tide begins at the middle and extends both ways (**2**). The flower itself consists of a white tube, which is divided at its end into four purple lobes. Of these four lobes the lowest is a trifle longer than the others and turns up slightly at its tip. Alternating with the lobes are four stamens, inserted on the tube of the corolla. A much reduced calyx, which encircles the base of the corolla, adheres to the inferior ovary. This typical epigynous flower is further characterized by a four-leaved calyx-like involucel, which invests the ovary and fruit (fig. 14.) The individual flowers begin their development as axial outgrowths of the bracts besetting the capitulum. This undifferentiated mass of cells, somewhat rounded at first, soon broadens a little, and the distinct lobes of the corolla appear on the peripheral portion (fig. 10). Next in appearance are the primordia of the stamens, which form the succeeding inner cycle (fig. 11). Soon after this the tissue below the lobes of the corolla and stamen initials begins to elongate en masse, forming a tubular ring. Following so soon that it would almost seem to be simultaneous, is the appearance of a twofold lateral swelling just below the base of the corolla (fig. 11). The upper protuberances are the primordia of the calyx and the lower develop into a calyx-like involucel, which later invests the ovary and fruit (figs. 11, 14). The last to make their appearance are the carpel lobes, which appear as basal outgrowths from the inner surface of the tubular cavity (fig. 12).



FIGS. 3–14.—Fig. 3, longitudinal section of young floral head showing origin of another head (daughter branch) in axils of clasping leaf; fig. 4, daughter floral head enlarged showing initials of involucral bracts; *t*, trichome included to show relative size of floral head at time of origin; fig. 5, later stage of floral head showing the clasping leaf; figs. 6, 7, still later stage showing initials of individual floral bracts; fig. 8, young floral head in longitudinal section; fig. 9, papilla of single flower; fig. 10, young flower showing origin of corolla; fig. 11, later stage showing beginning of stamens, calyx, and involucel; fig. 12, beginning of carpels; figs. 13, 14, later stages; abbreviations: *fh*, floral head; *cl*, clasping leaf; *ib*, involucral bract; *fb*, floral bract; *fp*, floral papilla; *cor*, corolla; *s*, stamen; *c*, calyx; *in*, involucel; *st*, style; *o*, ovule; *ca*, carpel.

Comparisons

In its departure from the order of succession of floral parts as exemplified by *Cnicus arvensis* (1) and *Silphium* (7), namely, corolla, stamens, carpels, and calyx, *D. sylvestris* is not unique. Although not exactly agreeing with HANNAH'S (4) report for *Galium Aparine* and *Valeriana officinalis*, in which the order of succession of floral parts is petals, stamens, sepals, and carpels, it is in perfect accord with the account of MARTIN (6) for *Aster* and *Solidago*, who describes the calyx in those forms as arising somewhat earlier, in fact almost simultaneously with the stamens. On the other hand, HANNAH states that the sepals of *Helianthus annuus* appear

TABLE I

Genus	Order of succession of floral parts				Investigator
Sambucus	Sepals	Petals	Stamens	Carpels	4
Galium	Petals	Stamens	Sepals	Carpels	4
Valeriana	Petals	Stamens	Sepals	Carpels	4
Dipsacus	Petals	Stamens	Sepals almost simultaneous with stamens	Carpels	Figs. 9–14, this paper
Fevillea	Sepals	Petals	Staminodia	Carpels	5
Solidago and Aster	Petals	Stamens	Sepals almost simultaneous with stamens	Carpels	6
Silphium	Petals	Stamens	Carpels	Pappus	7
Helianthus	Petals	Stamens	Carpels and sepals simultaneous		4
Cnicus	Petals	Stamens	Carpels	Pappus	1

about the same time as the carpels. A perfect acropetal succession, namely, sepals, petals, stamens, and carpels, is reported by HANNAH for *Sambucus canadensis*, and also by KIRKWOOD (5) for *Fevillea cordifolia*, etc.

Table I shows the succession of floral parts of these genera, members of closely related families.

It is seen that, at least so far as the forms in which the sepals are much reduced or modified are concerned, the order of succession of floral parts is uniform for petals and stamens only. Since the primordia of either sepals or carpels may be third in appearance or even simultaneous, it is evident that a more extensive study of epigyny is necessary before any safe conclusion can be reached.

Summary

1. The mode of branching is uniform for each individual plant.

2. New branches arise from the axils of clasping leaves.

3. The capitulum, or floral head, which terminates each branch is the first to make its appearance in the development of a branch.

4. The primordia of the bracts forming the involucre of the capitulum appear early, followed quickly by the initials of clasping leaves.

5. A twofold region of elongation sets in immediately above and below the initials of the clasping leaves of the new branch.

6. Secondary branches appear very early in the axils of the leaves clasping new branches.

7. The initials of the floral bracts appear before the papillae of the individual flowers, which they subtend.

8. The flowers are arranged in the form of a spiral.

9. The order of succession of floral members is corolla, stamens, calyx, and carpels, the calyx appearing almost simultaneously with the stamens.

10. The initials of a calyx-like involucel investing the ovary appear shortly after the initials of the calyx.

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RESERVE FOOD MATERIALS IN VEGETATIVE TISSUES

GWYNETHE M. TUTTLE

In view of the importance of the distribution of starch and fats as food reserve substances in vegetable tissues, and its connection with investigations regarding the effect of low temperatures on cells, it was thought desirable to investigate the conditions prevailing in some trees and shrubs of northern Alberta. Observations by several investigators have been made from other regions in the north temperate belt, such as those of LIDFORSS (3), MIYAKE (4), and SINNOTT (5) from Sweden, Japan, and eastern United States respectively. All of these districts lie between the winter isotherms of 30° and 40° F., whereas northern Alberta lies between those of 10° and 20° F. (1). Furthermore, vegetation in this region is frequently subjected to short periods of very low temperatures during the winter months, reaching −50° F., which makes the problem of resistance to cold a very important one.

LIDFORSS found that all "winter green" leaves are free from starch, but contain sugar and sometimes oil in winter. The starch is replaced by sugar during November, while the reverse change takes place in April. These results were largely confirmed for this region. Much of the material examined by SINNOTT retained starch as a food reserve throughout the winter, although many of the species were characterized by an oily reserve. He found that starch was most common in regions remote from the conducting channels, and fat most abundant in and near the phloem, close to the vessels. His "starch" trees were characterized by thick, squarish medullary ray cells with strongly lignified and small pitted walls; while the "fat" trees showed medullary ray cells with thin or unlignified walls and large pits. This he interpreted as indicating that "the character of the food reserve in any cell depends primarily upon the ease with which water, or substances carried by water, have access to the cell. Where the movement is apparently slow and difficult, the reserve persists as

starch; where such movement is easy, starch disappears at the beginning of winter and fat is produced."

A representative number of trees, shrubs, and perennial herbaceous plants of the region round Edmonton were examined. As the number of native plants with "winter green" leaves was very limited, tests of the stems of the deciduous types were included in the observations. Sections of the leaf, stem, and bud were tested with iodine solution and osmic acid at intervals from October to June. Records extending over three seasons show very little variation during October. Starch was quite abundant in the medullary ray cells, phloem, and cortex. Oils and fats were present in nearly all cells of the phloem and cortex and in the medullary ray cells of some plants. In several cases cortical cells contained food reserve which did not react to either iodine or osmic acid, suggesting the "transitory substance" mentioned by SINNOTT. Various tests were applied, but the identity of the substance was not determined. Some of the material was tested microchemically for sugar by means of the Flückiger reaction (**6**). Heavy precipitates of cuprous oxide were obtained (on heating) in *Syringa*, *Populus*, *Prunus*, *Salix*, *Shepherdia*, *Ribes*, *Picea*, *Pinus*, *Rosa*, *Pyrola*, *Cornus*, and *Eleagnus*, indicating the presence of glucose and dextrins. A positive determination of sugars could not be obtained by this means, on account of the possibility of the presence of a large number of other reducing substances in the cells; and in the absence of a satisfactory microchemical test for sugars. the work was not continued. Quantitative determinations of the sugar contents of a few cell saps made in another investigation have been recorded (**2**), where it was found that the total sugars varied from 0.5 to 2 per cent. The condition of the starch and oil reserve, tested at different seasons, is shown in table I.

In the majority of cases starch disappeared from the local plants during October and early November. Oils and fats were abundant in all of the species examined, with the exception of *Lonicera* and *Crataegus*. In view of the fact that a few species showed a trace of starch in their tissues quite late in winter, anatomical examinations were made and the two types described by SINNOTT

as "fat" and "starch" trees were recognized. Those species which retained small quantities of starch corresponded in all cases to the structure of "starch" trees, whereas those in which conversion was most rapid were of the "fat" tree type. Although species which retained definite starch reserve during the winter are absent from this locality, certain of the facts seemed to give limited support to the

TABLE I

Material	October	December	February	May
Populus tremuloides	S and O	O	O	S and O
Populus balsamifera	S and O	O	O	.....
Salix rubra	S and O	O	O	.....
Shepherdia canadense	S and O	O	O	S and O
Betula subcordata	S and O	.....	O	.....
Amelanchier alnifolia	S and O	.....	O	S and O
Alnus incana	S and O	.....	O	S and O
Pyrola rotundifolia	SS and O	O	O	O
Cornus canadensis	O	O	O	O
Linnaea borealis var. americana	SS and O	O	O	O
Mitella nuda	SS and O	O	O	.....
Corylus rostrata	S and O	.....	O	S and O
Picea canadensis	SS and O	O	O	.....
Pinus Banksiana	O	O	O	.....
Ledum groenlandicum	SS and O	.....	.....	S and O
Arctostaphylos Uva-ursi	O	O	O	S and O
Vaccinium Vitis-Idaea	SS and O	.....	.....	.....
Viburnum pauciflorum	S and O	O	O	S and O
Prunus pennsylvanica	S and O	O	O	.....
Ribes setosum	S and O	SS and O	SS and O	S and O
Symphoricarpos occidentalis	S and O	SS and O	SS and O	S and O
Rosa arkansana	S and O	SS and O	SS and O	S and O
Eleagnus argentea	S and O	SS and O	O	S and O
Cornus stolonifera	S and O	SS and O	O	S and O
Crataegus tomentosa	S and O	SS and O	O	S and O
Lonicera glaucescens	S	S	.....	S
Exotic shrubs				
Syringa vulgaris	S and O	SS and O	O	SS and O
Carragana sp	S	SS	No starch	S
Ribes sp	S and O	SS and O	O	.....
Acer Negundo	S and O	SS and O	SS and O	.....

S, starch; SS, slight starch reaction; O, oils and fats.

view recently suggested by SINNOTT in regard to the relation of structure to the nature of food reserve.

Tests were made of the leaves of the majority of the local deciduous trees and shrubs at the time of leaf fall. The 15 species examined were characterized by the absence of starch, except in

Ribes and *Cornus*, which were found to contain minute quantities, whereas all showed a relatively high oil and fat content. The winter buds of the same trees showed a high percentage of starch at this time. It would seem that the starch had either been converted in the mature leaves before leaf fall or else translocated to other regions of the plant. Table II records the leaf material tested.

TABLE II

Material	Food reserve
Symphoricarpos sp.	Oils and fats
Symphoricarpos occidentalis	Oils and fats
Ribes sp.	Oils and fats
Ribes setosum	Trace of starch
Betula subcordata	Oils and fats
Rubus sp.	Oils and fats
Corylus rostrata	Oils and fats
Fragaria sp.	Oils and fats
Corydalis sp.	Oils and fats
Ledum groenlandicum	Oils and fats
Arctostaphylos Uva-ursi	Oils and fats
Vaccinium Vitis-Idaea	Oils and fats
Syringa vulgaris	Trace of starch
Cornus stolonifera	Oils and fats

Several species of Salicaceae and Ericaceae were examined from high elevations in the mountains of Alberta and British Columbia, with a view to determining any difference in food reserve due to the different habitat. Herbarium specimens were used for the tests as fresh material was not obtainable. As these had been quickly and carefully dried, there is no reason to suppose that the starch or fat content of the cells would have been affected. Material of the stem was softened in water and tested immediately. Very definite reactions were obtained. Most of the material had been collected at the height of the vegetative season for the elevations at which it occurred. It was not possible to establish any connection between high elevation and a difference of food reserve. The majority of the species examined showed the presence of both starch and oil during the vegetative season, although a few contained only oil, as shown in table III.

TABLE III

MATERIAL	ELEVATION IN FT.	DATE OF COLLECTION
Starch and oil present		
Salix glaucops	6000	August 9
Salix nivalis	7500	July 18
Salix herbacea	8200	July 23
Phyllodoce empetriformis	7200	July 12
Kalmia glauca	5000	August 25
Phyllodoce glanduliflora	7500	July 18
Arctostaphylos Uva-ursi	5000	July
Ledum groenlandicum	5000	July
Rhododendron albiflorum	7500	July 27
Menziesia glabella	6000	July 3
Vaccinium scoparium	6500	June 26
Arctostaphylos alpina	Lowland	August 4
Arctostaphylos alpina	6700	June 25
Cassiope Mertensiana	4000	August
Oils and fats only		
Salix vestita	6400	July 17
Salix arctica	7100	July 26
Gaultheria ovatifolia	Lowland	August 15
Arctuous erythrocarpa	5000	August 15
Empetrum nigrum	5100	August 15

Summary

1. All the species examined showed a high starch content during the summer, which disappeared during October.

2. All the trees and shrubs of this district which were examined contained oils and fats as food reserve during winter with the exception of *Lonicera glaucescens* and *Crataegus* sp. The presence of sugar was demonstrated in many of the species. Quantitative determinations in a few places gave a total sugar content of 0.5–2 per cent.

3. Deciduous leaves, at the time of leaf fall, were devoid of starch, but contained oils and fats.

4. Most of the species of alpine Salicaceae and Ericaceae examined showed the presence of both starch and oil during the vegetative season. *Gaultheria ovatifolia*, a lowland species, showed only oil. Hence the ability to form starch does not seem to be associated with climatic conditions, resulting from high altitudes.

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BRIEFER ARTICLES

WILHELM PFEFFER

(WITH PORTRAIT)

WILHELM PFEFFER was born March 9, 1845, the son of a pharmacist, and died January 31, 1920. He studied at Göttingen, Marburg, Berlin, and Würzburg, taking his doctor's degree at Göttingen in 1865. He held university positions in Marburg, Bonn, Basel, and Tübingen before going to Leipzig in 1887, where he spent the rest of his life. It was here that he developed a laboratory and garden exactly according to his ideas, and this equipment probably held him at Leipzig in spite of calls elsewhere. PFEFFER probably shares with STRASBURGER the distinction of having more foreign students in his laboratory than any other German professor. His contribution to plant physiology, therefore, included not only his own researches, but also the stimulus of his ideas and methods to many productive students. He had what may be called an unusual perspective in connection with problems, seeing the various directions of attack, and the relations of results to the general field.

His publications, 96 in number, began with an ecological paper on mosses and some embryological papers, but soon passed into plant physiology. The sequence from the decomposition of carbon dioxide

by green plants, and the transformation of proteins during seed germination, to the investigation of tropisms and osmosis is familiar to all botanists. The influence of this work on osmosis extended beyond the botanical field into physics and chemistry. Of more general interest is his *Handbuch der Pflanzenphysiologie*, a notable reference text, the second edition of which was translated into English by EWART.

A sketch of PFEFFER's scientific career has appeared in *Science* (**51**:291. 1920), so that I shall attempt to supplement this by the presentation of some personal traits. When known to the writer PFEFFER lived in the second story of the Botanical Institute in the University Botanical Garden. He was a tall man and spare, with black hair and beard, and with a pleasing and kindly face that immediately put one at ease in meeting him. The portrait accompanying this sketch makes him look more austere than was his wont, otherwise it represents him as he looked in 1892, at the age of 48.

PFEFFER was a hard and continuous worker, and was rarely absent from the Institute, except for the four to six weeks' vacation which he generally took with his family in August and September in Switzerland or on the Baltic. He arose at six in the morning, lectured at seven, worked in his own rooms until eleven, then made the rounds of his students' tables, gave the noon hour to visitors, had luncheon, and worked again until five. From five to six he walked, and generally worked again in the evening. Besides keeping himself informed on the progress of science, especially physics and chemistry, he was always on the watch for new apparatus and new mechanical devices. Leipzig maintained a continuous mechanical and electrical exposition, and this PFEFFER frequently visited. He delighted in contemplating every new machine.

PFEFFER's attitude toward his students was friendly and cordial. Regularly he visited the research laboratories at eleven, discussed with each man his problem, designed apparatus, and pointed out lines of experiment, frequently calling in the first assistant to receive direction for providing what the worker needed. If any student needed more time than could be given in the forenoon visit, the Professor would come again in the afternoon. The first assistant was always conversant with the work of each student, and made the rounds forenoon and afternoon, besides always being on call when any help was needed. Each research man was given a key admitting him to the laboratory, and no restrictions were placed on his coming or going. Under PFEFFER's rule work had to make progress. There was patience, but there was

insistence on continuous work. Although students, especially the German students, treated the *Geheimrath* with the greatest deference, PFEFFER opened the way to the greatest freedom of intercourse, and the *Botanischer Abend*, held twice a month in some small room of a hotel, and attended by staff and research students, was notably a social occasion, giving opportunity for lively discourse and debate.

As was the lot of many other intellectuals in Europe, PFEFFER's last days fell upon evil times. The bare necessities of life were difficult to obtain; his only child, a son of 34 years, who had attained some prominence as a chemist, was killed in battle near the close of the war; the end of his professorship was near, by the rules of the new government; his country had fallen from her commanding position. The burden was too much for his sensitive soul, and it crushed him. Yet his life was successful beyond that of most men. There are few fields in plant physiology that have not been extended by his researches. Physics, chemistry, and general biology have profited by his classical monographs. His pupils are professors and teachers over the whole world.—F. C. NEWCOMBE, *University of Michigan, Ann Arbor, Mich.*

CURRENT LITERATURE

BOOK REVIEWS

Hydrogen ion determination

Hydrogen ion determinations are of ever increasing interest and importance to investigators in physiological and other fields of botany. Two methods of making such determinations are more or less widely known and well standardized: the electrometric and colorimetric methods. Both the history of the development and accounts of the practical operation of making such determinations have heretofore existed in scattered periodicals, and a student desiring to follow one of the methods had the choice of searching through several periodicals for the necessary information or of consulting such works as MICHAELIS.[1] A book by CLARK[2] has recently appeared which will be welcomed by students of hydrogen ion concentration, both on account of its detailed information and authoritative nature, written as it is by one who has made many contributions toward the development of methods for measurements of this sort. Chapter i deals with "General relations among acids and bases"; then follow 59 pages on the colorimetric determination of hydrogen ion, 108 pages on electrometric measurements, a short chapter of 5 pages on "Supplementary methods," and lastly a chapter of 29 pages on "Applications." The book also includes a very complete bibliography and an appendix containing tables for converting P_H to hydrogen ion concentration, other tables applicable to P_H measurements, and a list of representative potentiometer equipment.

Under "General relations among acids and bases" the author discusses the theory of dissociation in solution, and develops the related equations. The following 59 pages on colorimetric determinations include a chapter on the general method, two chapters on the theory and choice of indicators, another chapter devoted to buffer solutions, and two chapters concerning errors of the method and practical determinations with indicators. The author includes tables showing Sorensen's indicators, Clark and Lubs series, and Gillespie's drop-ratio series, giving their P_H ranges and other data necessary in using them. The section dealing with electrometric measurements is divided into ten chapters. They include a short outline of the method, the theory of the hydrogen electrode, discussion and illustration of various types of electrode vessels, potentiometers and other electrical apparatus, hydrogen generators

[1] MICHAELIS, LEONOR, Die Wasserstoffionenkonzentration. Berlin: Julius Springer Co. 1914.

[2] CLARK, W. MANSFIELD, The determination of hydrogen ions. pp. 317. Baltimore: Williams & Wilkins Co. 1920.

and temperature control, discussion of reduction potential and its relation to hydrogen electrode potential, sources of error, standard solutions for checking such measurements, and a chapter on P_H standardization, the last including a "Proposal of standard values." The chapter on "Supplementary methods" deals with the conductivity and other methods which have been used in special cases in estimating hydrogen ion concentrations. The final chapter on "Applications" groups the literature according to subject matter, and according to the author constitutes an index to the bibliography which follows.

The reviewer was especially interested in the details which the writer has added from his experience in the manipulation of apparatus. Among these are the charging of storage batteries using an ordinary electric light current, the effects of various substances (carbon dioxide, phenol, toluol, oxygen, etc.) in solution upon the hydrogen electrode, purification of mercury, construction of a constant temperature box, and lists and discussion of possible potentiometer equipment. For the student already engaged in making hydrogen ion determinations the book contains many valuable suggestions, and forms a ready reference to literature, while to the student who contemplates making such measurements it represents a manual, an outline of procedure.—J. M. ARTHUR.

A chemistry of plant products

HAAS and HILL'S, *An introduction to the chemistry of plant products*, which first appeared in 1913 and was reviewed in this journal,[3] has now reached a second edition.[4] From its first appearance it has been recognized as a book filling a long felt want. It was intended to supply the botanist, and especially the plant physiologist, with chemical knowledge and methods not found in the ordinary textbooks on chemistry. It has filled its purpose admirably. Although written primarily for the plant physiologist, the avoidance of a technical method of treatment makes it a useful book for the botanist of limited chemical training, who is working on problems involving a knowledge of plant materials. Thus at the present time, when the synthetic viewpoint of botany in relation to the other sciences is coming to the front, when it is coming to be recognized that botany and chemistry must unite forces in solving many problems, the method of treatment used in the book has increased significance. It is a significant commentary on the amount of work being done by WILLSTÄTTER and his coworkers, and by others no doubt inspired by them, that the major changes in the book have been made in the chapter on pigments. This chapter has been entirely rewritten and much new material incorporated, especially in the part dealing with chlorophyll. A few other important additions have been made to the text, and a number of references have been added

[3] BOT. GAZ. **56**:343. 1913.

[4] HAAS, PAUL, and HILL, T. G., An introduction to the chemistry of plant products. 2d ed. pp. xii+411. London: Longmans, Green. 1917.

to the literature, all serving to bring the work up to date. It will continue to be a book which the plant physiologist and anyone interested at all in the chemistry of plant materials will want on his shelf.—S. V. EATON.

Soil alkali

HARRIS[5] has written an excellent critical little book on soil alkali. The author says: "It has been estimated that about 13 per cent of the irrigated land of the United States contains sufficient alkali to be harmful. This means that there are over 9,000,000 acres of land under present canal systems that are affected with alkali. There are many more million acres of alkaline land in the United States that do not lie under irrigation systems. Similar figures might also be given for other countries of this continent and for all other continents. The alkali problem is one of no mean importance to farmers, nor to any who are interested in the world's food supply.

In a strictly chemical sense the word alkali refers to a substance having a basic reaction. As applied to the soil, however, this restricted meaning does not hold, and alkali refers to any soluble salts that make the soil solution sufficiently concentrated to injure plants. This includes the chlorides, sulphates, carbonates, and nitrates of sodium, potassium, and magnesium, and the chloride and nitrate of calcium. The sulphate and carbonate of calcium are not sufficiently soluble to be injurious to crops. Most of the alkalies are in reality neutral salts. It may be somewhat unfortunate to use for general substances a word that has become so well established in agricultural literature that it would now be very difficult to change it." The author also emphasizes the great number of purely scientific problems connected with alkali soils and the need of much fundamental research in this field.

The book includes 16 chapters: 1. Introduction; 2. Geographical distribution; 3. Origin of alkali; 4. Nature of alkali injury to plant; 5. Toxic limits of alkali; 6. Native vegetation as an indicator of alkali; 7. Chemical methods of determining alkalis; 8. Chemical equilibrium and antagonism; 9. Relation of alkali to physical conditions in the soil; 10. Relation of alkali to biological conditions in the soil; 11. Movement of soluble salts through the soil; 12. Methods of reclaiming alkali lands; 13. Practical drainage; 14. Crops for alkali land; 15. Alkali water for irrigation; 16. Judging alkali land.—WM. CROCKER.

NOTES FOR STUDENTS

Vegetation of Tasmanian mountains.—In reporting in some detail a study of the mountain vegetation of Tasmania, Miss GIBBS[6] sketches the position of the geological history of the island that most directly concerns its

[5] HARRIS, F. S., Soil alkali, its origin, nature, and treatment. pp. xvi+258. Wiley & Sons. 1920.

[6] GIBBS, L. S., Notes on the phytogeography and flora of the mountain summit plateaux of Tasmania. Jour. Ecol. **8**:1–17, 89–117. 1920.

present vegetation. The present area of 27,000 sq. mi. seems to have been much reduced during the latest glacial period, subsequent to its separation from Australia, now 184 miles distant. Its vegetation during that period consisted probably of moss and low shrubs only. As its present configuration comes from the dissection of one huge plateau, there are within the island no important barriers to migration, and the elevations do not exceed 5000 ft. The annual rainfall varies from 112 to 165 inches, while high winds are incessant upon the more elevated portions.

The *Eucalyptus* forests of the lowlands, the mixed forest of the west coast, and the vegetation of the tablelands and mountains constitute the three main plant formations of the island. These formations with their main subdivisions are briefly characterized, but only the higher elevations are considered in detail, and their vegetation is regarded as austral-montane rather than alpine. The higher plateaus range from 3500 to 4000 ft. in altitude, with a few rock masses higher. There are no glaciers or permanent snow fields, although during the winter months the mountains are often snow-covered, and this, together with heavy rains during the rest of the year and persistently high winds, constitutes a fairly rigorous climate, which results in a vegetation that is shrubby and spreading in habit, with small coriaceous leaves, and almost wholly without herbaceous forms except on the highest peak, where the snow remains late in the season. There a mosaic of low mosslike plants is developed, the individuals often taking the form of cushions.

Shrub associations dominate the more exposed plateau summits. Here the vegetation reaches a height of 1–1.5 m., and is decidedly xerophytic in aspect, showing rigid branching, small evergreen leaves, and often terminal flower clusters. These shrub associations vary from a very scattered display upon broken rock to dense masses with a well developed undergrowth where soil conditions are more favorable. Usually there is no massing of a single species, but several mingle freely. In one situation the endemic *Microcachrys* formed a dense green carpet for yards around well isolated groups of *Diselma Archeri*, *Podocarpus alpina*, *Coprosoma nitida*, and *Olearia pinifolia*. Other abundant genera are *Orites*, *Richea*, *Bauera*, *Epacris*, and *Helichrysum*. At somewhat lower altitudes the shrubs pass into the dwarf montane forest, one type of which consisted of trees like *Phyllocladus aspleniifolius*, *Arthrotaxis selaginoides*, *A. laxifolia*, and *Atherosperma* crowded together with shrubby *Diselma*, *Orites Milligani*, *Fagus Gunnii*, *Drimys aromatica*, *Telopea*, *Tetracarpaea*, and *Richea pandanifolia*, all about 2 m. in height. In more sheltered situations these forests reach a height of 3–5 mi., and may pass to *Eucalyptus* scrub.

The conditions of low temperature, intense illumination, with high winds and heavy rainfall, here limited to high altitudes, in the antarctic region are found at sea level and result in similar vegetation; hence it is not inappropriate to apply the term "antarctic" to this montane flora. The practical absence of annual leaf fall, and the entire absence of leguminous plants which act as

nitrifying agents, are considered responsible for the lack of progressive improvement of soil conditions and the persistence of xerophytes. The same factors account for the relative absence of herbaceous plants. In seeking for the origin of this flora, after an examination of the available evidence, Miss GIBBS concludes that the mountains of New Guinea may be considered as the focus of development and distribution of the so-called "antarctic" plants, justifying the term Papuan austral-montane for this group, of which, even on the limited basis of our present knowledge, nearly one-half of its most characteristic genera are now known from New Guinea. The author also contends that the northwesterly poleward wind which sweeps persistently over the mountains of New Guinea above tree level, in a constant direction and at a constant altitude, decreasing in height in its progress southward, is the agency by which this flora has been transported. Once established, the elements remain within the radius of the lower but equally constant circumpolar wind.

Collections from these montane associations show 108 species of vascular plants, of which 67 are endemic, the most remarkable family being the Coniferae with 7 genera and 9 species, 3 genera and 8 species being endemic. Other large families are the Proteaceae with 8 species, all endemic, the Myrtaceae with 5 species, 3 being endemic, the Epacridaceae with 20 species, of which 16 are endemic, and the Compositae with 19 species, 12 being endemic. Among families well represented in boreal montane regions, but much less conspicuous in Tasmania, are the Cyperaceae, Ranunculaceae, Cruciferae, Rosaceae, and Ericaceae, each represented by only a single species.—GEO. D. FULLER.

Aluminum and soil acidity.—MIRASOL[7] has done a piece of work on the relation of aluminum to soil acidity, working on three different acid silt loams from southern Illinois. "In the absence of some calcium compounds as a source of calcium, aluminum salts were highly toxic to sweet clover when applied in amounts chemically equivalent to five times the acidity of the soil. In the presence of calcium silicate, aluminum nitrate was more toxic than aluminum sulphate. . . . . Aluminum mono-hydroxide did not have any effect on sweet clover when other plant food elements were added in the soluble form. Calcium carbonate in sufficient amount corrected the toxicity of aluminum salts, by precipitating aluminum as calcium aluminate, an insoluble compound. Acid phosphate applied at the rate of 400 pounds per acre reduced the toxicity of aluminum salts by forming aluminum phosphate, an insoluble compound." Like HARTWELL and PEMBER, in an article recently reviewed in this journal, MIRASOL found that acid phosphate precipitates soluble aluminum, but in contrast to these investigators he found that acid phosphate decreases the acidity rather than increases it as they had assumed. "The form of aluminum immediately concerned in the unproductivity of acid soils in the soluble form is the salts. . . . . In soils sufficiently provided with calcium,

[7] MIRASOL, J. J., Aluminum as a factor in soil fertility. Soil Science 10:153–193. 1920.

toxic aluminum salts may never be found, but in soils deficient in calcium and other bases, as in acid soils, toxic aluminum salts are largely the end products of sulphofication and nitrification. It is not denied that iron and manganese may become contributing factors in the unproductivity of some acid soils, but the preponderance of evidence points to aluminum as the determining factor in the acidity of the soils under investigation."—WM. CROCKER.

Soluble substances in soils.—MCCOOL and MILLAR[8] have studied the rate at which substances become soluble in soils of various origins, types, ages, etc. The solubility was determined by the Bouyoucos freezing point method. The soils were leached free from soluble materials and then allowed to stand in water for various periods (5, 10, 30, and 60 days), and the freezing points determined at the ends of these periods. Contrary to the common view, soils from regions of lower precipitations are not more soluble than those from higher precipitations. The so-called new soils are less active than those somewhat older, and aged soils are almost inert. Subsoils liberate soluble salts very slowly, there being little activity below 6 inches. Sand particles are very inactive, and silts and clays are mainly responsible for the soluble materials. Grinding increases solubility. When soils were treated with 0.1N $NaNO_3$ and then washed free of soluble materials, the rate of dissolving was measurably affected. Western soils responded somewhat more readily than others. The Michigan Agricultural Experiment Station is studying this problem from several angles (composition of the soil, solutions on cropped and uncropped soils, residuary effects of salts on soils of different texture) and the work promises to be a valuable contribution to our knowledge of soil fertility. —WM. CROCKER.

Dormancy in trees.—COVILLE[9] has emphasized the significance of cold in forcing trees out of their autumn dormancy. He finds temperatures of 32–40° F. the most effective, and emphasizes the transformation of starches to sugars as an important result of the low temperature. The effective temperatures agree well with those found for the after-ripening of dormant embryos in this laboratory.—WM. CROCKER.

Living stumps.—The continued growth of stumps and the healing over of the cut surface is not uncommon in the case of *Pseudotsuga*. PEMBERTON,[10] investigating the phenomenon on Vancouver Island, British Columbia, finds the continued growth of the stumps due to the transference of food from living trees connected with stumps by means of subterranean root grafts. Instances are cited when growth ceased immediately with the cutting of the host tree.—GEO. D. FULLER.

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[8] MCCOOL, M. M., and MILLAR, C. E., The formation of soluble substances in soils taken from widely separated regions. Soil Science 10:219–235. 1920.

[9] COVILLE, F. V., The influence of cold in stimulating the growth of plants. Nat. Acad. Sci. 6:434–435. 1920.

[10] PEMBERTON, C. C., Living stumps of trees. Amer. Forestry 26:614–616. *figs.* 6. 1920.

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This book (third edition, completely revised) contains directions for collecting and preparing plant material for microscopic investigation. Killing and fixing, freehand sectioning, the paraffin method, the Venetian turpentine method, the celloidin method, and the glycerin method are treated in detail, and chapters on making photo-micrographs and lantern slides have been added. In later chapters specific directions are given for making such preparations as are needed in a study of the plant kingdom from the algae up to the flowering plants. Ten years' experience with the Venetian turpentine method has brought it to a high degree of efficiency; the paraffin method has been greatly improved; the notes on staining have kept pace with cytological research, and the directions for finding material are more extensive.

The book may be used in classes under an instructor, but the directions are so explicit that students working alone should soon learn to make first-class preparations.

With the exception of the sections on the glycerin and celloidin methods, this third edition is practically a new book.

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